size" and (3) "*it can be stated that the polysulphated polysaccharides xylosan sulphate and chondroitin polysulphate, but not heparin, improved aggrecan synthesis*" [emphasis added]. (See, respectively, abstract for (1) and (2) and conclusions for (3).)

In addition, the examiner, in citing Verbruggen, page 1669, 1st column, 5th paragraph refers to the production of hyaluronan by chondrocytes. These experiments were not performed on chondrocytes but on dedifferentiated chondrocytes. Furthermore it is noted that no heparin was used to test hyaluronan synthesis. Verbruggen indeed suggested ("*seem to be*") that polysulphated polysaccharides have a common effect on fibroblasts like cells, and that such effect also "*likely occurred*" on chondrocytes.

The penultimate paragraph on page 1670 explains that hyaluronan acts as a backbone to improve the accumulation of aggrecans. Thus although it is suggested that all polysulphated polysaccharides stimulate hyaluronan synthesis, the relevance of such enhanced hyaluronan synthesis is limited when no aggrecan synthesis takes place. Accordingly the relevant parameter to consider for chondrocyte developments is aggrecan synthesis rather than hyaluronan synthesis. The fact that heparin does not stimulate aggrecan synthesis indeed clearly teaches away from its use in chondrocyte development.

Furthermore, Verbruggen, at the *Material and Methods* on page 1665, left column, under the heading "*The polysaccharides*" discusses that both heparin and chondroitin have 2 to 3 sulphate groups per disaccharide. Despite the similar level of sulphatation, these two compounds are structurally different enough to have a different effect on aggrecan synthesis. This illustrates that the sulphation of a polysaccharide as such does not guarantee aggrecan synthesis and that its effect on aggrecan is not predictable. This leads Verbruggen to the conclusion that "*selected classes*" of polysulphates illustrate repair promoting effects. Which selected classes these are, is not disclosed or suggested. As is discussed below the non-thrombogenic property of a polysulphated polysaccharide does not motivate to consider such compounds.

In addressing Applicant's rebuttal of Verbruggen and Rosenberg, the Advisory Action states:

> Applicant argues that one of ordinary skill in the art would have not been motivated to combine Verbruggen and Rosenberg teachings and that the combination of references fails to provide a motivation to use polysulphated alginate for chondrocyte cultivation (see Remarks page 3, fourth paragraph filed on *05/04/2010*). These arguments are considered but are not found persuasive because Rosenberg et al. teach polysulfates prepared from alginic acid (polysulphated alginate) and chondroitin polysulphates are polysulfated polysaccharides, which are non-thrombogenic (heparin-like effect). Rihova teach a matrix suitable for implantation, must be non-thrombogenic (anticoagulant activity) to be biocompatible.

And the Office concludes:

> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to try (choosing form a finite number of identified polysulfated polysaccharides) and to use polysulfated polysaccharide, polysulphated alginate, in the method and the composition as taught by Verbruggen et al. with a reasonable expectation of success in providing an in vitro method for cultivation of chondrogenic cells, a composition comprising polysulphated alginate, and a method of treatment of cartilage defects. The motivation as taught by Verbruggen et al. would be stimulation of the production of high molecular weight hyaluronan by chondrocytes seems to be a common effect of polysulfated polysaccharides.

Accordingly, the Office maintains the obviousness rejection in view of Rosenberg (describing the anticoagulant properties of certain polysaccharides) and Verbuggen (describing the stimulation of aggrecan synthesis by certain polysaccharides). This combination is made in view of Rihova which discusses the biocompatibility of biomaterials.

Rihova comments on solid polymeric materials used as implants and soluble polymeric materials used as drug delivery systems. It is submitted that the section on soluble polymeric materials is not relevant for the present invention which relates to chondrocyte implants for cartilage repair. Rihova refers to the blood compatibility and

plasma protein adsorption of solid polymeric materials in respectively item 2.3 and 2.4. (last paragraph of page 161 to second paragraph of page 164). As is apparent for the skilled person, and also indicated in this section, the occurrence of thrombogenic events is only relevant to polymers which come into direct contact with the bloodstream.

Item 2.3.1 on complement activation states that "The complement system ... may be activated after a contact of the foreign material with blood" and discusses mainly membranes for dialysis.

Item 2.3.2. relates to platelet function and discusses catheters and artificial pancreas.

Item 2.3.3. relates to plasma coagulation and discusses ventricular assist devices.

Item 2.4 relates to plasma protein adsorption and discusses synthetic ligaments.

Applicant agrees that the concerns on blood coagulation, as expressed in Rihova, may be relevant for implants which are in intimate contact with blood, such concerns are irrelevant in the context of cartilage repair. Cartilage is an avascular tissue which does not come into contact with the bloodstream. Accordingly, the problems as raised in Rihova do not occur when a matrix with chondrocyte cells is implanted in a cartilage defect.

In addition, before the use of cellular implants, cartilage repair was performed by the so-called microfracture technique wherein small holes were made in the subchondral bone. This resulted in the release of bone marrow which contains chondrocyte precursors. These precursor cells differentiated into chondrocytes to generate new cartilage. The blood clot which is locally formed in the microfracture technique creates a fibrin network wherein the chondrocyte cells originating from the bone marrow can grow. The presence of a locally formed blood clot is thus considered as a beneficial side effect promoting cartilage repair.

Accordingly, blood coagulation is a process which is irrelevant to assess the compatibility of polymeric biomaterials in cartilage repair, and consequently the caveats

that are expressed in Rihova are not applicable in the claimed application.

Accordingly the statement of the Examiner that "*Rihova teach that a matrix must be non-thrombogenic to be biocompatible*" is not correct for the matrices used for cartilage repair as claimed in the present invention.

To support this position, Applicant directs the Examiner's attention to (1) Steadman J.R et al. (2001) *Clin. Orthop. Rel. Res.* **391S**, S362-369 (hereafter "Steadman."), (2) Sledge et al (2001) *Clinics. Sport Medicine.* **20**, 365-378 (hereafter "Sledge"); and (3) Mc Pherson J.M. and Ross T. (2000) in "Principles of tissue engineering, second edition", Lanza, RP., Langer, R. and Vacanti J. (Eds) Acad. Press, pp. 697-709 (hereafter "McPherson"). Each of these references documents that a blood clot provides a environment which favors cartilage repair. Copies of Steadman, Sledge, and McPherson are provided herewith.

Steadman

Steadman provides a detailed description of the microfracture technique, wherein perforations are made in the joint to release bone marrow components from the underlying subchondral bone, including blood, which form a clot that provides an environment for the generation of cartilage. In particular, Steadman, in the Abstract, states:

> Specially designed awls are used to make multiple perforations, or microfractures, into the subchondral bone plate. Perforations are made as close together as possible, but not so close that one breaks into another. They usually are approximately 3 to 4 mm apart. The integrity of the subchondral bone plate must be maintained. **The released marrow elements (including mesenchymal stem cells, growth factors, and other healing proteins) form a surgically induced super clot that provides an enriched environment for new tissue formation.** The rehabilitation program is crucial to optimize the results of the surgery. **It promotes the ideal physical environment for the marrow mesenchymal stem cells to differentiate into articular cartilagelike cells,** ultimately leading to development of a durable repair cartilage that fills the original defect [emphasis added].

The fact that blood is released from the subchondral plate is documented on page S364, end of the right column:

> The microfracture holes then are made toward the center of the defect (Fig 4). When the arthroscopic irrigation fluid pump pressure is reduced, under direct visualization **the release of** marrow fat droplets and **blood** from the microfracture holes into the knee can be observed (Fig 5) [emphasis added].

An explicit reference to the formation of a blood clot is found on page S366 right column, second paragraph:

> The microfracture technique produces a rough surface in the subchondral bone to which the marrow clot can adhere more easily, yet the integrity of the subchondral plate is maintained for joint surface shape. In addition to eliminating thermal necrosis and **providing a roughened surface for blood clot adherence**, the different angles of arthroscopic awls available provide easier access to difficult areas of the knee. The awls not only provide perpendicular holes but also provide improved control of depth penetration. **The key to the entire procedure is to establish the marrow clot to provide the optimal environmental for the body's own pluripotential marrow cells (mesenchymal stem cells) to differentiate into stable tissue within the lesion** [citations omitted][emphasis added].

Sledge

Sledge describes the release of blood and the subsequent formation of a blood clot. Sledges emphasizes that the blood clot should not be damaged, which provides further evidence that blood clot formation at the cartilage injury is a desired property for the skilled person in the field of cartilage repair.

Sledge, at page 6, second and third paragraph states:

> The bed, once completely punctured, is debrided gently of loose fragments of bone and soft tissue with a full-radius shaver. The full-radius blade has smooth edges that more precisely cut tissue (including articular cartilage) with less fraying than the more aggressive blades with teeth. At this point, the tourniquet can be taken down, and the **treated defect can be seen to fill with blood and subsequent clot** [emphasis added].

In the event other procedures are required in the same knee during the same operation, it is best to perform the microfracture last so as to avoid any clotting of the holes before adequate bleeding is allowed. Furthermore, once the tourniquet and inflow pressures are released, **the defect clot is forming, making it imperative that the joint is handled carefully while placing dressings, to avoid damaging the fragile clot** [emphasis added].

And, Sledge at page 10, third paragraph states:

Protection of the immature clot is the mainstay of postoperative care following microfracture, with avoidance of damaging axial, torsional, and shear stress to the defect. Controlled stress is applied to the healing fibrocartilage with continuous passive motion to contour the immature clot and subsequent fibrocartilage to the opposite joint surface, and improve the quality of the repair tissue [citations omitted][emphasis added].

Mc Pherson

McPherson describes on page 697, last paragraph and on page 698, first paragraph methods to penetrate subchondral bone to release bone marrow components and blood, and shows that the technique was well known the skilled person around 2000. McPherson states:

[S]urgeons developed several methods to penetrate the subchondral tissue (Fig 49.1) with the objective of enabling mesenchymal stem cells from the bone marrow to migrate into the wound site and mediate the repair process.

* * * *

Microfracture involves use of an awllike device to poke holes through the subchondral plate , and drilling involves arthroscopic drilling of holes through the subchondral plate. **Both procedures provide access of blood** and marrow-derived cells **to the defect site** [emphasis added].

Although McPherson does not explicitly mention the formation of the blood **clot,** it is clear that is the purpose of the procedure that blood is released during this procedure and that the artisan will observe the blood clot during the intervention.

The above argumentation on the article of Rihova demonstrates that no

obviousness objection can be made on a combination of prior art on aggrecan stimulating compounds (Verbruggen) and on compounds with anticoagulant activity (Rosenberg). Moreover, it is submitted that there was no motivation to consider polysulphated alginates as a candidate compound and that the skilled person could not predict its effect on chondrocyte development. The Office has failed to make a prima facie case of obviousness and the rejection should be withdrawn.

CONCLUSION

In view of the foregoing remarks, Applicant submits that the application is now in condition for allowance and such action is respectfully requested.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 9/7/2010

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Microfracture: Surgical Technique and Rehabilitation to Treat Chondral Defects

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Full-thickness articular cartilage defects rarely heal spontaneously. Some patients may not have clinically significant problems from chondral defects, but most eventually have degenerative changes. Techniques to treat chondral defects include abrasion, drilling, autografts, allografts, and cell transplantation. The senior author (JRS) developed the microfracture technique to enhance chondral resurfacing by providing a suitable environment for new tissue formation and taking advantage of the body's own healing potential. Microfracture has been done in more than 1800 patients. Specially designed awls are used to make multiple perforations, or microfractures, into the subchondral bone plate. Perforations are made as close together as possible, but not so close that one breaks into another. They usually are approximately 3 to 4 mm apart. The integrity of the subchondral bone plate must be maintained. The released marrow elements (including mesenchymal stem cells, growth factors, and other healing proteins) form a surgically induced super clot that provides an enriched environment for new tissue formation. The rehabilitation program is crucial to optimize the results of the surgery. It promotes the ideal physical environment for the marrow mesenchymal stem cells to differentiate into articular cartilagelike cells, ultimately leading to development of a durable repair cartilage that fills the original defect.

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Articular cartilage defects rarely heal spontaneously[2,4,11–17] regardless of whether the defects are acute, chronic, or degenerative. Although some patients do not have clinically significant problems from articular cartilage defects, with time most eventually will have degenerative changes associated with the cartilage damage.[3,4,17] These degenerative changes are progressive. They often become irreversible with development of profound arthritis if there is no adequate therapeutic intervention. Based on estimates, 32 to 37 million Americans of all ages have degenerative arthritis that has become progressive from either acute or chronic articular cartilage injuries.[14] Many of these patients become progressively disabled, and total joint arthroplasty may be the only alternative for pain relief.[4,17]

Despite the seemingly sudden interest in chondral defects, physicians have attempted to heal damaged or degenerative articular cartilage for more than 250 years. Many techniques have been used including spongialization, abrasion, drilling, tissue autografts, allografts, and cell transplantation.[1–4,9,11] Recently, clini-

cians have taken a greater interest and a more aggressive approach toward articular cartilage problems because of better understanding of cartilage biology and pathophysiology and of advances in imaging techniques and arthroscopic surgery. Attitudes have changed toward articular cartilage resurfacing; greater emphasis now is placed on it.[2–4,11,17]

A procedure referred to as the microfracture technique has been developed by the senior author (JRS) to enhance chondral resurfacing by providing an enriched environment for tissue regeneration and by taking advantage of the body's own healing abilities.[1,11, 13–15] This procedure first was used approximately 20 years ago based on an instinctive feeling that it might provide certain advantages over other techniques such as drilling smooth round holes. The microfracture procedure[13–15] now has been used in more than 1800 patients.

The Microfracture Technique

Indications for Microfracture

The microfracture procedure was designed for patients with posttraumatic lesions of the knee that have progressed to full-thickness chondral defects. The general indication for microfracture is full-thickness loss of articular cartilage in either a weightbearing area between the femur and tibia or in an area of contact between the patella and trochlear groove.[1,11,13–15] Unstable cartilage that overlies the subchondral bone also is an indication for microfracture. Another indication is degenerative changes in a knee that has proper axial alignment. Although these changes may not be true osteochondral defects, they are in fact loss of articular cartilage at the bone-cartilage interface.

General considerations for use of the microfracture procedure include patient age, acceptable biomechanical alignment of the knee, and activity level. If all of these criteria define a patient who may benefit from chondral resurfacing, then such a patient should be considered for microfracture.

Contraindications for Microfracture

Contraindications for microfracture include axial malalignment (as described below), a patient unwilling to follow a strict and rigorous rehabilitation protocol, partial-thickness defects, inability to use the opposite leg for weightbearing during the minimal weightbearing time, and a relative contraindication for patients older than 60 years.[13] The age of 60 years is only a relative contraindication if the patient is able to meet all other criteria, but the authors have observed that some patients older than 60 years have difficulty with walking with crutches and the rehabilitation. Other specific contraindications include any systemic immune-mediated disease, disease-induced arthritis, or cartilage disease.

Two methods for radiographic measurement of the biomechanical alignment of the weightbearing axis of the knee are used: (1) the angle made between the femur and tibia on anteroposterior (AP) views obtained with the patient standing; and (2) the weightbearing axis drawn from the central portion of the femoral head to the center of the tibiotarsal joint on long standing radiographs. If the angle drawn between the tibia and femur is greater than 5° varus or valgus from the normal, this amount of axial malalignment would be a relative contraindication for microfracture. The weightbearing line preferably should be in the central ¼ of the tibial plateau of either the medial or lateral compartment. If the weightbearing axis falls outside the centralmost ¼ of the plateaus, medial or lateral, this weightbearing shift also would be a relative contraindication.

The Surgical Procedure: Microfracture

Three portals are made about the knee for use of the inflow cannula, the arthroscope, and the working instruments. A tourniquet is not used routinely. An initial thorough diagnostic examination of the knee is done. Routinely the authors carefully inspect the suprapatellar pouch, the medial and lateral gutters, the patellofemoral joint, the intercondylar notch and its contents, and the medial and lateral com-

partments including the posterior horns of both menisci. Typically, other necessary intraarticular procedures are done before doing microfracture, with the exception of ligament reconstruction. This routine helps prevent loss of visualization when the fat droplets and blood enter the knee from the microfracture holes. Additionally, particular attention is focused on soft tissues such as plicae and the lateral retinaculum that potentially could produce increased compression between cartilage surfaces.[5]

After assessing the full-thickness articular cartilage lesion, the exposed bone is debrided of all remaining unstable cartilage. To debride the cartilage, the authors use a full radius resector or Gator shaver (Linvatec Corporation, Largo, FL), a hand-held curved curette, or both. All loose or marginally attached cartilage from the surrounding rim of articular cartilage also is debrided to form a stable perpendicular edge of healthy viable cartilage around the defect (Fig 1). This prepared lesion provides a pool that helps hold the marrow clot, super clot as the authors have termed it, as it forms. The calcified cartilage layer that remains as a cap to many lesions then is removed by using a curette (Fig 2). Thorough and complete removal of the calcified cartilage layer is extremely important based on the authors' basic science research.[6] To avoid excessive damage to the subchondral bone, an arthroscopic awl (Linvatec Corporation) then is used to make multiple holes, or microfractures, in the exposed subchondral bone plate. The authors use an awl with an angle that permits it to be perpendicular to the bone as it is advanced. The 90° awl is advanced only manually, not with a mallet. The 90° awl typically is used only on the patella or other soft bone. The holes are made as close together as possible, but not so close that one breaks into another and damages the subchondral plate between them. This technique usually results in microfracture holes that are approximately 3 to 4 mm apart. When fat droplets can be seen coming from the marrow cavity, the appropriate depth (approximately 2 to 4 mm) has been reached. The arthroscopic awls produce essentially no thermal necrosis of the bone compared with hand-driven or motorized drills. Generally, the authors make microfracture holes around the periphery of the defects first, immediately adjacent to the healthy stable cartilage rim (Fig 3). The microfracture holes then are made toward the center of the defect (Fig 4). When the arthroscopic irrigation fluid pump pressure is reduced, under direct visualization the release of marrow fat droplets and blood from the microfracture holes into the knee can be observed (Fig 5). When the

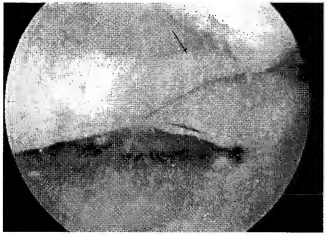

Fig 1. A full-thickness chondral defect on a femoral condyle that has been prepared for microfracture is shown. Damaged cartilage has been debrided to form a stable perpendicular edge of healthy cartilage (arrow).

Fig 2. A curette is used to remove the calcified cartilage layer of a full-thickness chondral defect.

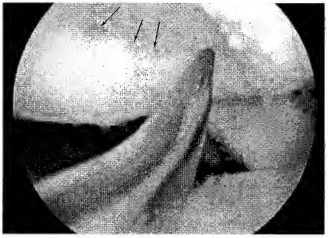

Fig 3. A chondral defect that has been debrided and is being microfractured is shown. The microfracture holes are started at the periphery of the defect adjacent to the stable cartilage (arrows).

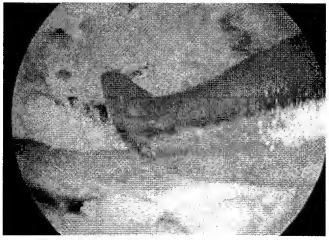

Fig 4. Microfracture holes are continued into the central portion of the defect. The microfracture awl is penetrating the subchondral bone approximately 2 to 4 mm in depth.

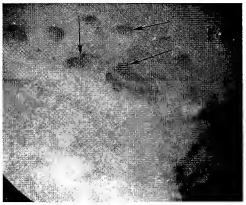

Fig 5. Marrow elements including blood and fat droplets (arrows) can be seen coming from the microfracture holes after the arthroscopic irrigation fluid pressure has been reduced.

quantity of marrow contents flowing into the joint appears adequate, all instruments are removed from the knee and the joint is evacuated of fluid. No intraarticular drains are placed because the goal is for the surgically induced marrow clot rich in marrow elements to form and to stabilize while covering the lesion.

It is common for the chronic degenerative chondral lesions to have extensive eburnated bone and bony sclerosis with thickening of the subchondral plate,[9] making it difficult to do an adequate microfracture procedure. In these instances and when the axial alignment and other indications for microfracture are met, the authors make a few microfracture holes with the awls to assess the thickness of the subchondral plate. The authors then use a burr to remove the sclerotic bone until punctate bleeding is seen. After the bleeding, a microfracture procedure can be done routinely. Results have improved noticeably for these patients with chronic chondral lesions since using this technique. If, however, the surrounding cartilage is so thin that it is not possible to establish a perpendicular rim to hold the marrow clot, then a microfracture procedure likely would not be done in patients with such advanced degenerative lesions (Fig 6).

The microfracture technique produces a rough surface in the subchondral bone to which the marrow clot can adhere more easily, yet the integrity of the subchondral plate is maintained for joint surface shape. In addition to eliminating thermal necrosis and providing a roughened surface for blood clot adherence, the different angles of arthroscopic awls available provide easier access to difficult areas of the knee. The awls not only provide perpendicular holes but also provide improved control of depth penetration. The key to the entire procedure is to establish the marrow clot to provide the optimal environmental for the body's own pluripotential marrow cells (mes-

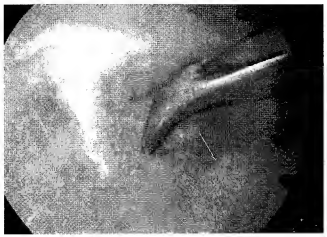

Fig 6. In this degenerative lesion with thinning of the surrounding cartilage, a perpendicular rim (arrow) still is available to hold the surgically induced marrow clot.

enchymal stem cells) to differentiate into stable tissue within the lesion.[16,13–15]

The authors emphasize to their patients that they likely will not start to experience improvement in their knees for at least 6 months after microfracture. Improvement can be expected to occur slowly and steadily for 2 years based on the authors' prior experience.

Rehabilitation After Microfracture

The rehabilitation program after microfracture for treatment of chondral defects in the knee is crucial to optimize the results of the surgery.[7,8] The rehabilitation program is designed to promote the ideal physical environment in which the newly recruited mesenchymal stem cells from the marrow can differentiate into the appropriate articular cartilagelike cell lines. The authors think that the surgically induced marrow clot provides the basis for the ideal chemical environmental to complement the physical environment. This cellular differentiation ultimately leads to the development and proliferation of a durable repair cartilage that fills the original defect.[1,6,13–15]

There are many factors to consider when formulating the postoperative rehabilitation program after microfracture. The specific protocol recommended depends on two essential factors, specifically, the anatomic location and the size of the defect. These factors are critical to determine the ideal postoperative plan.[7] For example, if other intraarticular procedures are done concurrently with microfracture, such as anterior cruciate ligament reconstruction, the authors do not hesitate to alter the rehabilitation program as necessary. A full discussion of all of the possible variations of the rehabilitation program is beyond the scope of the current study. Two protocols now will be described.

Rehabilitation Protocol for Patients With Lesions on the Femoral Condyle

Patients with lesions on the weightbearing surfaces of the femoral condyles are treated with a continuous passive motion machine commencing immediately in the recovery room.[1,10,13–15] The initial range of motion (ROM) is 30° to 70°, and then it is increased as tolerated by 10° to 20°, until full passive ROM is achieved. The rate of the machine usually is one cycle per minute, but the rate can be varied based on patient preference and comfort. Many patients tolerate use of the machine at night. For those who do not, however, the authors' experience indicates that intermittent use during the day is equally beneficial. Regardless, the goal is to have the patient in the continuous passive motion machine for 6 to 8 hours every 24 hours. If the patient is unable to use the continuous passive motion machine, then instructions are given for passive flexion and extension of the knee with 500 repetitions three times per day. Full passive ROM of the injured knee is gained as soon as possible after surgery.

The authors also prescribe cold therapy for all patients postoperatively. The authors' experience and observations indicate that the cold helps control pain and inflammation, and most patients state that the cold provides overall postoperative discomfort relief. These empirical observations are similar to findings reported by Ohkoshi and coworkers[10] when cold therapy was used after anterior cruciate ligament reconstruction. Cold therapy usually is used for 1 to 7 days postoperatively.

Crutch-assisted touchdown weightbearing ambulation is prescribed for 6 to 8 weeks, depending on the size of the lesion. For most patients, 6 to 8 weeks seems an adequate time to limit weightbearing. However, for patients with small lesions (< 1 cm diameter), weightbearing may be hastened by a few weeks. Patients with lesions on the femoral condyles or tibial plateaus rarely use a brace.

Limited strength training also begins immediately after surgery. Patients do double leg ½ knee bends the day after surgery.[7] Because they are touchdown weightbearing, patients place most (75% to 80%) of their body weight on their uninjured leg to do the exercise. Stationary biking without resistance and a deep water exercise program are begun at 1 to 2 weeks after microfracture. The deep water exercises include use of a kick board and a flotation vest for deep water running. After 8 weeks, patients progress to full weightbearing and begin a more vigorous program of active motion of the knee. Elastic re-

sistance cord exercises are begun at approximately 8 weeks after microfracture. A detailed description of use of the cord and the exercises has been published previously.[7] The ability to achieve predetermined maximum levels for sets and repetitions of elastic resistance cord exercises is an excellent indicator for progressing to weight training. Free or machine weights are permitted when the patient has achieved the early goals of the rehabilitation program, but not before 16 weeks after microfracture. The authors strongly emphasize the importance of proper technique when beginning a weight program.[7] Depending on the clinical examination, it may be recommended that patients do not return to sports that involve pivoting, cutting, and jumping until at least 4 to 6 months.

Rehabilitation Protocol for Patients With Patellofemoral Lesions

All patients treated by microfracture for patellofemoral lesions must use a brace set at 0° to 20° for at least 8 weeks. This brace limits compression of the regenerating surfaces of the trochlea, the patella, or both. Passive motion is allowed with the brace removed, but otherwise the brace must be worn at all times. Patients with patellofemoral lesions are placed into a continuous passive motion machine immediately postoperatively. Cold therapy also is used. The regimen aims for the patients to obtain a pain-free and full passive ROM soon after surgery.

For patients with patellofemoral joint lesions, joint angles are observed carefully at the time of arthroscopy to determine where the defect comes into contact with the patellar facet or the trochlear groove. These areas are avoided during strength training for approximately 4 months. This avoidance allows for training in the 0° to 20° range immediately postoperatively because there is minimal compression of these chondral surfaces with such limited motion.

Patients with lesions of the patellofemoral joint treated by microfracture are allowed weightbearing as tolerated, but it must be limited to the angles of knee flexion where the lesion is not compressed. Therefore, it is essential for patients to use a brace that prevents placing excessive shear force on the maturing marrow clot in the early postoperative period. The brace routinely is locked between 0° and 20° ROM to prevent flexion past the point where the median ridge of the patella engages the trochlear groove. After 8 weeks, the knee brace is opened gradually before it is discontinued. When the brace is discontinued, strength training is advanced progressively.

Potential Complications of Microfracture

Most patients progress through the postoperative period with little or no difficulty. Some, however, present with mild transient pain, most frequently after microfracture in the patellofemoral joint. Small changes in the articular surface of the patellofemoral joint may be detected by a grating or gritty sensation of the joint, particularly when a patient discontinues use of the knee brace and begins normal weightbearing through a full ROM. Patients rarely complain of pain at this time, and this grating sensation typically resolves spontaneously in a few days or weeks.

Similarly, if a steep perpendicular rim was made in the trochlear groove, patients may notice catching or locking as the apex of the patella rides over this lesion during joint motion. Some patients may even perceive these symptoms while in the continuous passive motion machine. It has been the authors' experience that these symptoms usually dissipate within 3 months. If this perceived locking is painful, then the patient is advised to limit weightbearing and avoid the symptomatic joint angle for an additional period.

Occasionally, a recurrent effusion develops between 6 and 8 weeks after microfracture, usually when a patient begins to bear weight on the injured leg after microfracture of a femoral condylar defect. This effusion may mimic the preoperative effusion, except usually it is painless. This type of painless effusion is treated conservatively. Typically, it resolves within several weeks after onset. Rarely has a second arthroscopy been required for recurring effusions.

Future Considerations

There are important future considerations for chondral resurfacing. As the orthopaedic community continues to gain a better understanding of the biology of articular cartilage, orthopaedic surgeons must identify and understand endogenous biologic modulators of healing within the joint. Efforts should continue to examine the exogenous application of various factors to influence the cellular response and cartilage healing. The sciences of tissue engineering and gene therapy and the use of synthetic matrices are also likely to be critical to future success. Orthopaedic researchers must continue to attempt to gain a better understanding of the key role played by the calcified cartilage layer and the subchondral bone in the formation of chondral defects and in cartilage healing.

The advantages of the microfracture include that less heat, and therefore less necrosis, is produced than with drilling. The microfracture awls allow access to virtually the entire joint, whereas access is much more limited when using a drill. Furthermore, selection of the correctly angled awl permits the microfracture holes to be made perpendicular to the surface of the subchondral plate, whereas in most cases drilling is done at an angle not perpendicular to the bone. The roughend surfaces produced by the microfracture technique provide a surface to which the marrow clots can adhere firmly. Although the subchondral bone plate is penetrated, its actual integrity as a structure (and therefore joint contour and shape) is maintained. Perhaps most important, this technique provides access to biologic modulators of healing and to mesenchymal stem cells that have the ability to differentiate into cartilagelike cells and produce a durable repair cartilage.

References

1. Blevins FT, Steadman JR, Rodrigo JJ, et al: Treatment of articular cartilage defects in athletes: An analysis of functional outcome and lesion appearance. Orthopedics 21:761–768, 1998.
2. Brittberg M, Lindahl A, Nilsson A, et al: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895, 1994.
3. Buckwalter JA: Articular cartilage: Injuries and potential for healing. J Orthop Sports Phys Ther 28:192–202, 1998.
4. Cohen NP, Foster RJ, Mow VC: Composition and dynamics of articular cartilage: Structure, function, and maintaining healthy state. J Orthop Sports Phys Ther 28:203–215, 1998.
5. Cohen ZA, McCarthy DM, Roglic H, et al: Computer-Aided Planning of Patellofemoral Joint OA Surgery: Developing Physical Models from Patient MRI. In Wells WM, Colchester A, Delp S (eds). Medical Image Computing and Computer-Assisted Intervention. New York, Springer-Verlag 9–20, 1998.
6. Frisbie DD, Trotter GW, Powers BE, et al: Arthroscopic subchondral bone plate microfracture technique augments healing of large osteochondral defects in the radial carpal bone and medial femoral condyle of horses. J Vet Surg 28:242–255, 1999.
7. Hagerman GR, Atkins JA, Dillman C: Rehabilitation of chondral injuries and chronic degenerative arthritis of the knee in the athlete. Oper Tech Sports Med 3:127–135, 1995.
8. Irrgang JJ, Pezzullo D: Rehabilitation following surgical procedures to address articular cartilage lesions of the knee. J Orthop Sports Phys Ther 28:232–240, 1998.
9. Johnson LL: The Sclerotic Lesion: Pathology and the Clinical Response to Arthroscopic Abrasion Arthroplasty. In Ewing JW (ed). Articular Cartilage and Knee Joint Function: Basic Science and Arthroscopy. New York, Raven Press 319–333, 1990.
10. Ohkoshi Y, Ohkoshi M, Nagasaki S, et al: The effect of cryotherapy on intraarticular temperature and postoperative care after anterior cruciate ligament reconstruction. Am J Sports Med 27:357–362, 1999.
11. Rodrigo JJ, Steadman JR, Silliman JF, et al: Improvement of full-thickness chondral defect healing in the human knee after debridement and microfracture using continuous passive motion. Am J Knee Surg 7:109–116, 1994.
12. Singleton SB, Silliman JF: Acute chondral injuries of the patellofemoral joint. Oper Tech Sports Med 3:96–103, 1995.
13. Steadman JR, Rodkey WG, Briggs KK, et al: Die Technik der Mikrofrakturierung zur Behandlung von kompletten Knorpeldefekten in Kniegelenk. Orthopaede 28:26–32, 1999.
14. Steadman JR, Rodkey WG, Singleton SB, et al: Microfracture Procedure for Treatment of Full-thickness Chondral Defects: Technique, Clinical Results and Current Basic Science Status. In Harner CD, Vince KG, Fu FH (eds). Techniques in Knee Surgery. Media, PA, Williams & Wilkins 23–31, 1999.
15. Steadman JR, Rodkey WG, Singleton SB, et al: Microfracture technique for full-thickness chondral defects: technique and clinical results. Oper Tech Orthop 7:300–304, 1997.
16. Urrea LH, Silliman JF: Acute chondral injuries to the femoral condyles. Oper Tech Sports Med 3:104–111, 1995.
17. Walker JM: Pathomechanics and classification of cartilage lesions, facilitation of repair. J Orthop Sports Phys Ther 28:216–231, 1998.

MICROFRACTURE TECHNIQUES IN THE TREATMENT OF OSTEOCHONDRAL INJURIES

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OSTEOCHONDRAL INJURIES OF THE KNEE

MICROFRACTURE TECHNIQUES IN THE TREATMENT OF OSTEOCHONDRAL INJURIES

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In our quest to relieve musculoskeletal pain and suffering with its attendant loss of function, orthopaedists have made numerous attempts to restore the integrity of articular cartilage. Open surgical debridement with drilling of the defect had many advocates in the 1950s.[21] Clinical results were variable and not well documented. Many other means of achieving a resurfacing of the "exposed bone" of full-thickness chondral defects have been proposed over the years since. Periosteal and perichondral flaps,[18] cultured chondrocytes, [5] osteochondral allografts,[10] osteochondral autografts,[3] [19] total or partial joint replacements, fibrin clot stimulation,[20] growth factors,[13] osteotomy with or without any of the above,[7] [17] synthetic implants,[8] electrical stimulation, [6] and arthroscopic drilling, abrasion, and microfracture all have their advocates.[1] [9] The important factors to consider when assessing any method include patient morbidity (e.g., pain, stiffness, blood loss, time off work,

cosmesis), reproducibility of results, surgical time, cost, technical difficulty, and current clinical data supporting each method.

ALTERNATIVES TO MICROFRACTURE

Replacement of articular cartilage with soft tissue consisting of only periosteum has been studied mainly in the laboratory, with limited study in humans. The cambium layer cells adjacent to the bone interface are thought to be the source of chondrocytes. Histologic studies of repair tissue from periosteal flaps in animal studies have been promising, with a high content of type II collagen.[16] At this time, arthroscopic techniques are not available, and fixation to surrounding normal cartilage is a technical difficulty, with potential necrosis of cartilage surrounding the defect with suture.[4]

Costal or mandibular cartilage grafts of perichondrium have been used in animal studies to resurface chondral defects since the early 1970s.[11] Mandibular cartilage, unlike the articular cartilage in other joints, has the capacity to regenerate. One human study revealed approximately 90% of the defects in 27 patients filled with chondral tissue.[12] Another human study showed hyaline cartilage produced in 12 of 15 and fibrocartilage in 3 of 15 patients treated with perichondral grafts. The advantages of this technique include lack of immune reaction, low cost (no need to culture cells), and minimal morbidity of graft site if costal cartilage is used. No reports have been made to date of arthroscopic techniques, which would necessitate the use of an arthrotomy, thus increasing the morbidity.

Technology has made it possible to culture autologous chondrocytes in vitro over a 2- to 3-week period after either open or arthroscopic harvest. The expanded cell population then is implanted open after debriding unhealthy cartilage in the defect, suturing a periostial flap over the defect, and injecting the cells beneath the flap. The subchondral bone is not denuded to bleeding bone. Non-weightbearing is recommended for 2 to 3 months postoperatively. In one study, 73% had hyaline cartilage on biopsy at 24 months mean, and 27% had fibrocartilage.[5] Most recently, Petersen presented his first 100 patients treated with autologous cartilage cell expansion and transplantation, and reported between 75% and 96% good and excellent results with various sites of involvement. Results were based on only the absence of locking as the main determinant, however, with pain and swelling secondary. No formal knee scoring system was used. Therefore, other studies from unaffiliated centers are needed to confirm the results of Brittberg and Petersen. Currently, when interpreted

carefully, their results are not any better than those obtained with arthroscopic chondroplasty, and incur significant morbidity (arthrotomy, two-stage surgery, up to 3 months non-weightbearing) and a significant cost ($10,000 for the culture alone).

Osteochondral grafts have the advantage of placing hyaline cartilage directly into the chondral defect. Allografts pose the difficulty of immune rejection and must be contoured to fit the defect perfectly. Autograft has the advantage of no host reaction; however, repair is usually necessary to achieve coverage without leaving a sizeable defect at the donor site. The tissue that fills in between the cartilage-bone plugs is fibrocartilage. This technique seems to be particularly suited to osteochondritis dissecans because of the bone loss that occurs in this disease.[19]

Joint resurfacing with inert implants, such as total or unicompartment replacement, is reserved for the relatively inactive patient with disease on both sides of the involved compartments. Osteotomy for unloading an involved compartment can be used with a biologic resurfacing technique if malalignment is significant. Tibial osteotomy is applicable to varus malalignment with medial overload, and distal femoral osteotomy can be used for valgus malalignment with lateral overload. Finally, an unloader brace can be useful for malalignment situations, with biologic resurfacing as an alternative to osteotomy.[15]

Research has shown promise for other methods of stimulating chondrogenesis in areas of deficient articular cartilage. No human studies to date have been reported in the literature, and therefore animal studies are mentioned for completeness. All have shown the ability to produce various degrees of hyaline and fibrocartilage. An exogenous fibrin clot has been shown to improve healing compared to controls in dogs.[20] Defects were made into bleeding bone in both groups, but the clot was washed and blotted free in the control group. This study simply confirms our impression that fibrin clot, whether exogenous or produced by bleeding from the bone bed, will produce fibrocartilage.

An exciting method for regenerating cartilage in deficient areas is the use of synthetic or biologic matrices that contain chondrocytes grown in culture. If the matrix were adequately pliable and once in place could maintain structural integrity, it could allow earlier weightbearing than other techniques, and possibly be implanted with a mini-incision or arthroscopically.[8] Freed et al have shown reconstitution of the subchondral plate and adherance of the new cartilage to the surrounding host cartilage. Furthermore, growth factors could be added to the matrix, theoretically improving the cartilage development to a greater extent.

The efficacy of electrical stimulation for promotion of fracture healing has been well documented, and one study has shown beneficial effects on

chondrogenesis.[6] Multiple growth factors have been shown to stimulate chondrocyte mitotic activity, including transforming growth factor-beta (TGF-beta), fibroblast growth factor, insulin-like growth factor, and platelet-derived growth factor. Optimal doses and ratios still are being determined.

Drilling into the subchondral bone of a chondral defect allows marrow elements, including mesenchymal stem cells, growth factors, and fibrin, to enter the defect and form a clot that undergoes metaplasia to form fibrocartilage. Originally, drilling was promoted for treatment of osteoarthritis with an open technique, then gradually was adapted to arthroscopy. One of the theoretical disadvantages is the drill can burn the bone surrounding the perforation, and another is that it leaves a polished surface that is mechanically less able to adhere to the fibrocartilage. Also, it can be technically demanding to reach certain areas with a straight drill bit while maintaining a perpendicular orientation to the bone surface. K-wire drilling can overcome this somewhat because of the flexibility of the wire; however, this produces more polishing of the bone.

Abrasion arthroplasty for full-thickness chondral defects has been used during the last two decades.[14] Rather than perforating the subchondral bone in multiple locations, a burr is used to abrade the subchondral bone down to bleeding cancellous bone. This technique produces primarily fibrocartilage. The subchondral plate is destroyed, however, thus creating less subchondral stress protection and causing a thicker cap of fibrocartilage, theoretically lowering the biomechanical properties of the healed tissue. Finally, the depth of penetration is more difficult to control and a bed of bone filled with pits is often the result.

RATIONALE FOR MICROFRACTURE CHONDROPLASTY

The technique of microfracture of the subchondral bone for articular cartilage defects was created by J. Richard Steadman, MD, of Vail, Colorado. I am grateful that he taught me this technique and many other procedures in the knee, and for conveying his rationale for microfracture.

Other techniques of chondroplasty arthroscopically involve the use of power equipment at high speeds, with the potential to cause heat necrosis of bone and surrounding cartilage; however, microfracture is done with a cartilage awl designed to produce a small perforation of the bone without producing heat. Moreover, the subchondral plate is left intact, thus preserving the load-bearing cortical bone, and avoiding deepening the defect and further altering the biomechanical characteristics of the repaired site. Also, the perforations can be produced in a more controlled manner than with a drill or burr. The tip of the awl is relatively sharp, and can be placed into the bone slightly to assure the perforation occurs at the

desired spot, whereas a drill or burr can jump or skip into healthy surrounding cartilage. Cartilage awls are reusable, whereas most arthroscopic burr tips are not, thus effecting a cost savings over abrasion arthroplasty. A rough surface is created by the microfracture technique, which improves adherence to the fibrocartilage.

The 30°, 45°, and 90° angled tip of cartilage awls allow access to areas of the patella, trochlea, and posterior femoral and tibial condyles not accessible with a drill. These angles also facilitate maintenance of the desired perpendicular orientation of the perforations to the plane of the subchondral bone, which can be difficult with a straight drill or burr tip. Therefore, microfracture is technically attainable by the arthroscopist without special training, adds minimal cost to the procedure, and has low risk. Perhaps the more invasive or expensive and technically demanding procedures are useful in revision or in failed cases. Numerous studies have been published in recent years documenting the clinical effectiveness of microfracture technique combined with a specific postoperative protocol.[2] [22] [24]

SURGICAL TECHNIQUE

General Technique

Awls designed specifically for microfracture are available in varying angles from Linvatec Corporation, Florida. Arthroscopic portals are created with a drain cannula superolaterally underneath the vastus lateralus tendon, a viewing portal 3 to 4 mm proximal to the lateral joint line, and a working medial portal at the joint line. All articular surfaces are surveyed with the 30° scope and a standard probe. Any breaks in the surface are probed carefully to detect any flap tears or fissures down to bone. Flaps are assessed for degree of detachment, and a ring curette or full-radius shaver is used to debride the tissue back to a stable high vertical wall edge. The high wall is important to creating less shear stress on the clot within the defect that serves as the nidus of cells for the healing fibrocartilage. Care must be taken to assure that all remaining edges are well attached to the underlying bone.

For simple fissures (one line of vertical separation) without flap formation on either side of the fissure, the awl can be used to puncture the subchondral bone at the apices, and every 3 mm along the split. This is done to stimulate sealing of the fissure.

In a larger defect, the calcified cartilage layer must be removed down to, but not into, subchondral bone. This can be done with a closed curette, or a full-radius shaver with light manual pressure. Portals should be well

released, with regard to the synovial membrane medially and laterally, to facilitate entry of the sharp awl into the joint. The author places the arthroscope viewing angle in such a way to see the intraarticular opening of the working portal as the awl enters, so as to avoid inadvertent puncture of normal cartilage. The awl then is used to puncture the subchondral bone into the area of blood suppy below the cortex. Often, fat or marrow elements can be seen coming out of the "microfracture," confirming adequate depth of penetration. The punctures should be started at the periphery of the lesion and are made with the awl perpendicular to the subchondral plate. The holes should not be connected, a 1- to 2-mm bridge should be left between them at a minimum. Care should be taken to avoid undermining the healthy cartilage at the periphery of the lesion.

The bed, once completely punctured, is debrided gently of loose fragments of bone and soft tissue with a full-radius shaver. The full-radius blade has smooth edges that more precisely cut tissue (including articular cartilage) with less fraying than the more aggressive blades with teeth. At this point, the tourniquet can be taken down, and the treated defect can be seen to fill with blood and subsequent clot.

In the event other procedures are required in the same knee during the same operation, it is best to perform the microfracture last so as to avoid any clotting of the holes before adequate bleeding is allowed. Furthermore, once the tourniquet and inflow pressures are released, the defect clot is forming, making it imperative that the joint is handled carefully while placing dressings, to avoid damaging the fragile clot.

At the end of the procedure, one must determine what range of motion will cause contact of the defect with the opposite side of the joint. Thus, the surgeon can determine the minimum necessary range of continuous passive motion and the need for altered weightbearing status or bracing. Rehabilitation is undertaken with the premise that the healing defect is fragile and needs protection for 8 weeks postoperatively. Continuous passive motion (CPM) also is used for 8 weeks following surgery to improve the healing and durability of the fibrocartilage.[22] As an alternative, for patients without access to CPM, 500 knee motions in the contact range three times a day will suffice; however, this is less desirable, especially with patellar or trochlear groove defects, because it is, by necessity, at least partially active rather than passive.

TECHNIQUE BY SITE AND TYPE OF LESION

The knee is divided into three compartments with articular cartilage on each side. To discuss location-specific technique, the knee is divided into six distinct areas: medial femoral condyle (MFC), medial tibial plateau

(MTP), lateral femoral condyle (LFC), lateral tibial plateau (LTP), patella, and trochlear groove of femur (TG).

Medial Femoral Condyle

Visualization of the medial femoral condyle is best done by means of a lateral viewing portal with a medial working portal. Although some medial femoral condylar lesions are on the posterior condyle, most are on the distal end of the condyle and are best seen and approached with the knee flexed from 40° to 80°. Routine examination of the posterior condyle is good practice, however.

Debridement of the lesion is carried out in the usual fashion; however, care must be taken to avoid flattening the medial wall of the defect while shaving the medial defect edge. Two different methods can be used to accomplish this. First, moving the shaver from the medial to the lateral portal makes a more direct approach to the medial wall. Second, a ring curette with a curved neck can allow creation of a sharp edge of healthy cartilage while keeping the cut vertical to the bone plate.

The bed is punctured with the 45° awl through the medial portal. An assistant typically holds the arthroscope while the surgeon makes each microfracture with the awl and a tap or two with a mallet. The bed is debrided of loose fragments and the edges probed to confirm a stable junction with healthy cartilage. In the event a meniscectomy also is required, the author prefers to perform the meniscectomy first and the chondroplasty last to reduce the likelihood of deep clotting of the puncture holes medially before releasing the tourniquet and inflow pressure at the end of the procedure. This reduces the possibility of incomplete clot filling the defect at the end of the surgery.

Medial Tibial Plateau

Lesions of the medial tibial plateau are located most often along the lateral edge of the middle horn of the medial meniscus. Visualization is best obtained with the knee flexed 30° to 60° and valgus applied against a commercial leg holder to open the medial compartment. The author places the arthroscope in the lateral viewing portal, and uses a standard medial portal for instrumentation, as this avoids working over the top of the tibial eminences.

Debridement of the lesion bed is similar to other areas; however, the anterior edge is at risk of losing its high vertical wall with the shaver alone. Therefore, a curved shaver or curette is used to facilitate its maintenance, along with increased knee flexion to improve the angle of attack of the instruments.

Microfractures are created with the 45° awl in most cases; however, more

anterior portions of the lesions can require a 90° awl to allow maintenance of a perpendicular approach to the surface. Removal of loose fragments is performed with the shaver in the medial portal.

Lateral Femoral Condyle

In general, lesions of the lateral femoral condyle can be more difficult to visualize initially during survey arthroscopy, and great care must be taken to view the entire condyle. If overlying synovium or fat pad obscures portions of the condyle, it needs to be resected. The best visualization is from the lateral portal, with the working portal medial for most lesions; however, placing the scope medially and working from lateral can improve margin protection in some cases. The knee usually is placed in a figure-of-four position with flexion from 60° to 100° because most lesions are more posterior than the medial femoral condyle.

Debridement of the calcified cartilage layer and any remaining articular cartilage is facilitated by excising the infrapatellar plica off the roof of the intercondylar notch. This allows less restriction of movement of instruments crossing the joint from the medial side. Cartilage removal is easiest with a curved curette or shaver. As with the medial condyle, the medial edge of the defect can be flattened by the shaver, and if this appears to be occurring, the scope and shaver portals must be switched.

Punctures are customarily made with a 45° awl from medial, maintaining a perpendicular approach to the subchondral plate in each area of the lesion by altering knee flexion as necessary. More posterior lesions can be accessible only with the 90° awl, and more anterior lesions are easiest approached with the 30° awl from the lateral portal.

Lateral Tibial Plateau

Lesions of the lateral plateau can be difficult to access; fortunately, they are less common. The author uses a slightly higher lateral viewing portal in all cases (as taught by J. Richard Steadman, MD), allowing placement of a portal inferior to the lesion for working instrumentation in cases of bucket handle meniscal tears and chondral work, especially laterally. Flexion angles range from 45° to 80° with a figure-of-four position, and often a moderate amount of pressure by the assistant on the medial femoral condyle.

Remaining articular cartilage is removed with a shaver medially, after removing the infrapatellar plica and any excess synovium or fat pad anterolaterally. Knee flexion angle will facilitate the approach to the various areas of the defect. A 45° awl is used for the entire lesion in many cases; however, the 90° awl can be useful posteriorly. With lateral lesions, it is particularly useful to place the awl into the intercondylar notch area

with the knee in neutral varus-valgus position, then gradually advance it into the lateral conpartment under direct vision as the assistant places the knee into a figure-of-four position.

Patella

Patellar chondral defects most often are located in the central and lateral facets, and require careful probing to avoid underdiagnosis of deep damage. The high lateral portal (mentioned in the lateral tibial plateau section) is particularly useful for patellar viewing; often, placing the scope in the superolateral drain portal will improve visualization and diagnostic accuracy. Most patellar work is done easiest with the knee in full extension, and can be facilitated further by lateral retinacular release when indicated. Working portals for patellar procedures vary with the location and often are switched throughout the procedure. The author often uses a superomedial or midmedial portal for instrumentation in addition to the standard three portals. Positioning of the patella by the assistant also can facilitate access.

Lesion debridement is performed in the usual fashion, but curved shavers can be a tremendous advantage. Use of 90° awls is preferable in patellar defects, but the awls must be sharp, because the subchondral bone is often dense. It is often necessary to move the scope to another viewing portal after the microfracture is thought to be complete, to confirm an adequate puncture in all locations of the defect.

Alternatively, a mini-arthrotomy can be performed for difficult patellar lesions, using a Senn retractor to lift up the patella, and performing the chondroplasty and microfracture open. Skin, retinaculum, and capsule are incised from 2 to 3 cm on the side of the lesion, as determined arthroscopically. Visualization is improved with a headlight or highly focused operating room light. Microfracture is performed similarly to the arthroscopic technique, with a standard closure.

Femoral Trochlear Groove

As with the patella and lateral condyle, visualization of the femoral trochlear groove can be difficult, and the entire trochlear groove must be examined carefully. Longitudinal splits are often down to bone and should be treated carefully with microfracture to avoid damaging the edges of normal cartilage, if they are still adherent to bone. Flaps are often present medially and laterally in fissures that initially appear to be simple. Often, the synovial fat pad interferes with adequate visualization, and at times can be loosened by resecting the infrapatellar plica without shaving the fat pad itself.

In the author's practice, trochlear groove lesions often are treated

aggressively if patients are having patellofemoral pain and even mild crepitance when found incidentally at arthroscopy for another injury, such as a meniscus tear. Even grade 3 lesions with flaps, or lesions with cobblestone cartilage tissue in the defect, are treated with resection of unhealthy cartilage and microfracture.

Access to the defect from the medial portal is best done with the knee in 0 to 45° of flexion. Excision of the calcified cartilage and flaps is best done with a full-radius shaver to define the edges of healthy cartilage, and then freshened or completed with a curved ring curette if the edges are not sharp. Microfracture then is carried out with the 45° awl, and the loose fragments removed with the shaver. Occasionally, the scope must be placed superolaterally or medially to facilitate creation of the high wall rim.

POSTOPERATIVE CARE AND REHABILITATION

Protection of the immature clot is the mainstay of postoperative care following microfracture, with avoidance of damaging axial, torsional, and shear stress to the defect. Controlled stress is applied to the healing fibrocartilage with continuous passive motion to contour the immature clot and subsequent fibrocartilage to the opposite joint surface, and improve the quality of the repair tissue.[18] [22] [24]

Non-weightbearing is required for defects of the femoral condyles and tibial plateaus for 8 weeks postoperatively because most lesions are in weightbearing areas. Patients with patellar and trochlear groove lesions without weightbearing condyle lesions are allowed to weightbear in a knee immobilizer. Contact of the defect with the opposite joint surface is negligible in this position while walking in an immobilizer, but must be confirmed intraoperatively by observing the range of motion at which contact occurs.

Continuous passive motion is prescribed for 8 weeks postoperatively, for 6 to 8 hours per day. Most patients use the machine at night while sleeping, and tolerate it well. Research clearly has shown its beneficial effects, both in animals and humans, following chondroplasty of full-thickness defects. In cases where CPM was not available, instructions were given for 500 repetitions of non-weightbearing range-of-motion exercises actively for condyle and plateau lesions and assisted with the contralateral leg for patellofemoral lesions three times a day. Patients are educated on the merits of both protected weightbearing and continuous passive motion preoperatively and postoperatively to improve compliance.

Rehabilitative exercise during the first 8 weeks (stage 1 rehab period) is limited by the need for protected stress, especially avoidance of shear and

torsional overload. Isometric exercises of the quadriceps and hamstrings are performed during the first 2 weeks postoperatively in full extension. Patellofemoral contact is minimal in this position and loading of femoral and tibial condylar lesions is not excessive. Electrical muscle stimulation can be used in cases with poor quadriceps recruitment. Stationary bicycle use by the nonoperated limb while resting the operated side on a stepstool or other low object is beneficial to maintain cardiovascular fitness. A toe strap for the pedal is required to complete the revolution by using hamstring contraction to pull the pedal into the upstroke.

From the second through eight weeks postoperatively, stationary bicycle or deep water exercise (AquaJogger, Eugene, OR) is allowed with minimal resistance at a slow rate (60 rpm or less). Patients with patellar and trochlear groove lesions are not allowed on the bicycle at all or in the pool with knee flexion angles that demand contact with the defect by the opposite joint surface during this period. Deep water exercise is possible with the aquajogger buoyancy belt using a straight-leg exercise program, however.

No open chain (leg extension) exercises with resistance are allowed at any point in the rehabilitation of patellofemoral lesions. Patellofemoral contact forces are excessive with this knee exercise and are discouraged for all patients regardless of pathology. Closed chain exercises with minimal resistance, such as leg press or Total Gym, are allowed for condyle and plateau lesions less than 1 cm in size from the end of the second week postoperatively through the eighth week. Unlike the weightbearing of walking, there is minimal shear and torsional stress and light axial stress. The Total Gym is particularly useful in low angles of inclination and with use of the arm pulleys. This places most of the stress on the upper extremities to unload the knee, and at lower angles of inclination reduces the overall weight to be resisted during each cycle. For lesions of the condyles and plateaus over 1 cm, the author often will allow light resistance closed chain exercises after the sixth postoperative week.

Stage 2 rehabilitation is from the end of the eighth week to the end of the fourth postoperative month. During this time, emphasis is placed on regaining a normal gait pattern and resistance training. Although regaining quadriceps tone, girth, and strength is critical, one must also specifically strengthen the hamstrings, gastrosoleus, and hip musculature, and regain aerobic condition. Lunges are specifically avoided during this period for all lesion locations, along with ballistic exercises such as box-drills and plyometrics. Other high-impact aerobic exercise, such as stair running and sprints, are avoided for the first 3 to 4 months postoperatively in most cases, depending on lesion size and location.

An excellent rehabilitative tool during stage 2 rehabilitation is the Sport Cord. Low-impact forward-backward walking, progressing to running and

side-to-side agility drills are accomplished readily with the cord. Leg press, hamstring curls, hip flexor and extensor, and abductor and adductor strengthening exercises along with partial bilateral and eventually unilateral are allowed during this stage. The Sport Cord is available in varying resistances. It is portable, and therefore can be used at home, office, or gym, and while traveling. Furthermore, resistance can be altered simply by decreasing or increasing tension on the cord.

Lower extremity alignment is assessed during stage 2 rehabilitation, and adjusted if necessary. For patellofemoral lesions, hyperpronation is often present with lateral overload syndromes, and custom orthotics can be useful in unloading this area. Condylar lesions medially with some varus malalignment can benefit from lateral heel wedges, and medial wedges for valgus and lateral lesions. Osteotomy is considered in the active patient with severe malalignment in varus or valgus with a lesion in the overloaded compartment. Distal femoral osteotomy is used for severe valgus knees with either lateral femoral or tibial lesions or severe lateral patellofemoral lesions with instability. Proximal tibial osteotomy is indicated in some cases of severe varus malalignment with medial femoral or tibial defects.

Bracing is useful during stage 2 in all patellofemoral defects, and some condylar and plateau defects. Patellofemoral braces of neoprene with either a felt horseshoe that is adjustable, a J-shaped pad (Don Joy J-Strap), or Palumbo or air cell patellofemoral brace allow controlled pressure on the patella, thus unloading of the defect area somewhat. Pressure is applied to the contralateral side of the patellar defect.

In cases of varus or valgus malalignment that are passively correctable on examination, especially with the knee in 20° to 60° of flexion with condylar or plateau lesions, an unloader brace can protect the healed lesion. The author uses these for vigorous patients with lesions over 1 cm and malalignment. Varus malalignment is more easily braced than valgus, but the latter can be braced in many instances. Many brace manufacturers produce unloader braces; most are single hinged, on the side of the stress overload. Medial defects with varus, for example, have hinges placed medially with the oblique strap lateral to produce a valgus load to the joint.

Stage 3 rehabilitation is undertaken 3 to 4 months following surgery, and consists of progressive sports-specific training in the athlete, and increased aerobic and strength conditioning in the nonathlete. Total rehabilitation time following surgery is often 6 to 9 months. Some athletes are fully rehabilitated at 4 to 5 months postoperatively, and are allowed to return to sport if they can demonstrate the absence of effusions, pain, and point tenderness, with full muscle recovery and proprioception. Second-look arthroscopy is not routinely performed to assess degree of healing, but is reserved for patients with mechanical symptoms, recurrent

effusions, pain, or all three.

SUMMARY

Although many methods currently are available to treat articular cartilage lesions in the knee, microfracture has many inherent advantages. No other technique has been studied in the athletic population, and, in fact, most other procedures have been studied in the arthritic knee or in a mixed population of patients. Blevins et al have shown microfracture to be an effective tool in the treatment of chondral defects in both recreational and high-level athletes with mean follow-up of 3.7 years.[2]

Furthermore, microfracture is minimally invasive because it is arthroscopic through standard portals in most cases. The subchondral plate is preserved, unlike in other techniques, improving load-bearing characteristics following healing. No heat necrosis or polishing is introduced into the subchondral bone and marrow with microfracture. The depth of penetration and location of puncture is controlled readily with the various awl angles. Surgical equipment and supply costs are minimal, without the need for expensive cell cultures or nonstandard equipment. No harvest site morbidity is present, unlike osteochondral, perichondral, periosteal, or chondral autograft procedures. Microfracture is not overly demanding technically, but emphasis must be placed on meticulous handling of the subchondral plate and surrounding healthy cartilage and adequate debridement of unhealthy cartilage. Finally, a good surgical technique is only as good as its rehabilitation. Strictly emphasizing the need for compliance with weightbearing restrictions and the use of CPM is essential to successful patient rehabilitation.

References

1. Bert J, Maschka K: The arthroscopic treatment of unicompartmental gonarthrosis: A 5-year followup study of abrasion arthroplasty. Arthroscopy 5:25-32, 1989 Abstract

2. Blevins F, Steadman J, Rodrigo J, et al: Treatment of articular cartilage defects in athletes: An analysis of functional outcome and lesion appearance. Orthopaedics 21:761-768, 1998

3. Bobic V: Arthroscopic osteochondral aurograft transplantation in anterior cruciate reconstruction: A preliminary clinical study. Knee Surg Sports Traumatol Arthrosc 3:262-264, 1996 Abstract

4. Breinan H, Minas T, Hsu H, et al: Effect of cultured chondrocytes on repair of chondral defects in a canine model. J Bone Joint Surg Am 79A:1439-1451, 1997 Abstract

5. Brittberg M, Lindahl A, Nilsson A, et al: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889-895, 1994 Abstract

6. Buckwalter J, Mow V: Cartilage repair in osteoarthritis. *In* Moskowitz R, Howell D, Goldberg V, et al: Osteoarthritis: Diagnosis and Medical/Surgical Management, ed 2. Philadelphia, WB Saunders, 1992, pp 71-107

7. Buckwalter J, Mankin H: Articular cartilage: II. Degeneration and osteoarthritis, repair, regeneration and transplantation. J Bone Joint Surg 79A:612-632, 1997

8. Freed L, Grande D, Lingbin Z, et al: Joint resurfacing using allograft chondrocytes and a synthetic biodegradeable synthetic scaffolds. J Biomed Mater Res 28:891-899, 1994 Abstract

9. Friedman M, Berasi C, Fox J, et al: Preliminary results with abrasion arthroplasty in the osteoarthritic knee. Clin Orthop 208:141-145, 1986

10. Garrett J: Treatment of osteochondral defects of the distal femur with fresh osteochondral allografts. Arthroscopy 2:222-226, 1986 Abstract

11. Girdler N: Repair of articular defect with autologous mandibular condylar cartilage. J Bone Joint Surg 75B:710-714, 1993 Abstract

12. Homminga G, Bulstra S, Boumeester D, et al: Perichondral grafting for cartilage lesions of the knee. J Bone Joint Surg 72B:1003-1007, 1990

13. Hunziker E, Rosenberg L: Induction of repair in partial thickness articular cartilage lesions by a timed-release of TGF-beta. Trans Orthop Res Soc 19:236, 1994

14. Johnson L: Arthroscopic abrasion arthroplasty: Historical and pathological perspective: Present status. Arthroscopy 2:54-69, 1986 Abstract

15. Kirkley A, Webster-Bogaert S, Litchfield R, et al: The effect of bracing on gonarthrosis. J Bone Joint Surg 81A:539-550, 1999 Abstract

16. Kreider H, Moran M, Keeley F, et al: Biologic resurfacing of a major joint defect with a cryopreserved allogeneic periosteum under the influence of continuous passive motion in a rabbit model. Clin Orthop 300:288-296, 1994

17. Odenbring S, Egund N, Lindstrand A, et al: Cartilage regeneration after proximal tibial osteotomy for medial gonarthrosis. An arthroscopic, roentgenographic and histologic study. Clin Orthop 277:210-216, 1992

18. O'Driscoll S, Salter R: The repair of major osteochondral defects in joint surfaces by neochondrogenesis with autogenous osteoperiosteal grafts stimulated by CPM: An experimental investigation in the rabbit. Clin Orthop 208:131-140, 1986

19. Outerbridge K, Outerbridge A, Outerbridge R: The use of a lateral patellar autologous graft for repair of a large osteochondral defect in the knee. J Bone Joint Surg 77A:65-72, 1995 Abstract

20. Paletta G, Arnoczsky S, Warren R: The repair of osteochondral defects using an exogenous fibrin clot. An experimental study in dogs. Am J Sports Med 20:725-731, 1992 Abstract

21. Pridie K: A method of resurfacing osteoarthritic joints. J Bone Joint Surg 41B:618-619, 1959

22. Rodrigo J, Steadman J, Silliman J, et al: Improvement of full thickness chondral defect healing after debridement and microfracture using continuous passive motion. Am J Knee Surg 7:109-116, 1994

23. Shimuzu T, Videman T, Shimazaki K, et al: Experimental study on the repair of full thickness chondral defects: Effects of varying periods of continuous passive motion, cage activity and immobilization. J Orthop Res 5:187-197, 1987 Abstract

24. Silliman J, Steadman J, Rodrigo J, et al: Predictors of success in treating articular cartilage defects in the knee. Orthop Trans 16:724, 1992

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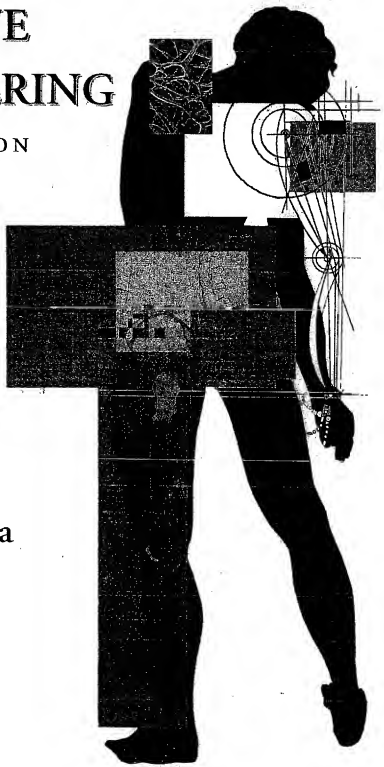

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PRINCIPLES OF TISSUE ENGINEERING

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CHAPTER 49

ARTICULAR CARTILAGE INJURY

John M. McPherson and Ross Tubo

INTRODUCTION

The fact that articular cartilage has little or no capacity for effective repair following traumatic injury has been recognized by clinicians for more than 250 years (Hunter, 1743). Even today natural history of the progression of joint degeneration following injury is not completely understood, but it is clear that injury to articular cartilage often leads to symptomatic pain and eventually to osteoarthritis (Dandy, 1991; Johnson, 1986b; Johnson-Nurse and Dandy, 1985). It appears that degeneration of articular cartilage following injury may be a consequence of an imbalance in the normally slow remodeling and maintenance processes provided by chondrocytes, which are imbedded in the dense extracellular matrix of the articular surface. The mechanisms responsible for tilting the balance of remodeling in favor of degradation, versus synthesis, are not understood, but may in part reflect local changes in biomechanical loading of chondrocytes following injury that profoundly influence cell metabolism.

It has been postulated that articular cartilage has a limited capacity for repair due to a limited supply of cells in the vicinity of the wound to mediate the repair process (for review, see Gillogly *et al.*, 1998). Unlike skin, for example, in which both vasculature and adjacent tissues provide cells for mediating the wound healing process, articular cartilage contains no vascular supply. In addition, articular chondrocytes, which are normally involved in articular cartilage synthesis and maintenance, are encased in a dense extracellular matrix that limits their mobility and their ability to contribute to the wound healing process. Synovial cells are present in synovial fluid, but apparently their numbers are too few or their biological properties are too limited to mediate adequate repair of any but the most minute cartilage defects (Hunziker and Rosenberg, 1996).

Numerous surgical procedures have been developed during the past 30 years in an effort to deal with the problem of cartilage injury, particularly following the advent of arthroscopic surgery. The most conventional approach to cartilage injury is debridement and lavage (Baumgaertner *et al.*, 1990; Bert and Maschka, 1989; Dandy, 1991; Rand, 1991; Timoney *et al.*, 1990). The primary objective of this procedure is to improve the contour of the cartilage defect by shaving the edges of the lesion and removing the cartilage debris generated either by the original injury or the debridement procedure. In general, clinical reports in the literature suggest that debridement and lavage provide immediate short-term relief of the symptoms in the majority of patients with small cartilage defects. The efficacy provided by the procedure fades with time, particularly in defects of greater than 1 cm^2, and many patients exhibit evidence of osteoarthritis, as judged by joint space narrowing.

Defects treated with debridement and lavage usually remain devoid of repair tissue unless the subchondral plate is penetrated during the debridement procedure. Based on this observation, surgeons developed several methods to penetrate the subchondral tissue (Fig. 49.1) with the objective of enabling mesenchymal cells from the bone marrow to migrate into the wound site and mediate the repair process. Abrasion arthroplasty involves the use of an arthroscopic burring device to remove tissue in the base of the wound to the level of subchondral bone (Bert and Maschka,

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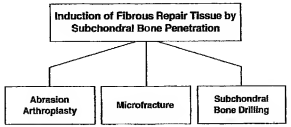

Fig. 49.1. Conventional surgical procedures utilized to treat damaged hyaline articular cartilage.

1989; Friedman *et al.*, 1984; Johnson, 1986a,b; Kim *et al.*, 1991). As with debridement and lavage, short-term results (i.e., less than 3 years) with this procedure appeared to provide satisfactory results in the majority of patients evaluated. Results were generally considered to be disappointing at time points in the time frame of 3 to 5 years, with only approximately 50% of the patients reporting satisfactory results. Alternative strategies for providing bone marrow-derived cells to the defect site have included microfracture and drilling (Buckwalter and Lohmander, 1994; Rodrigo *et al.*, 1994). Microfracture involves use of an awllike device to poke holes through the subchondral plate, and drilling involves arthroscopic drilling of holes through the subchondral plate. Both procedures provide access of blood and marrow-derived cells to the defect site. The clinical results using these procedures have reportedly been similar to those observed with abrasion arthroplasty, i.e., short-term efficacy but limited long-term utility (Gillogly *et al.*, 1998).

It is generally accepted that procedures that result in penetration of the subchondral plate lead to the formation of fibrocartilage. Fibrocartilage is composed primarily of type I collagen and proteoglycans such as versican, which are characteristic of mesenchymal tissues such as dermis. The extracellular matrix composition and organization of fibrocartilage provide significantly lower biomechanical compressive strength as compared to hyaline cartilage. The formation of fibrocartilage in articular defects following subchondral plate penetration is somewhat analogous to the formation of scar in skin following cutaneous injury. The main difference is that although the reduced biomechanical strength of scars in skin rarely leads to wound dehiscence, the reduced biomechanical properties of fibrocartilage in articular defects in most patients ultimately lead to failure of the repair tissue and degeneration of the surrounding tissue in the pathological process of osteoarthritis.

Articular cartilage, on the other hand, is normally composed of hyaline cartilage. Hyaline cartilage is composed of a complex organization of type II collagen and other minor collagens in combination with hyaluronic acid and a cartilage-specific proteoglycan termed aggrecan. Some of the differences in extracellular matrix composition between hyaline cartilage and fibrocartilage are summarized in Table 49.1. The hyaline cartilage-specific extracellular matrix molecules convey the capacity to withstand significant compressive forces without displaying significant deformation.

Table 49.1. Biochemical comparison of hyaline cartilage and fibrocartilage[a]

Hyaline cartilage	Fibrocartilage
Type II collagen	Type I collagen
Type VI collagen	Proteoglycans
Type IX collagen	Hyaluronic acid
Aggrecan	Link protein
Hyaluronic acid	
Link protein	
COMP protein	

[a]Key biochemical components that are useful for distinguishing hyaline articular cartilage from fibrocartilage. The specific biochemistry and organization of extracellular matrix molecules in these two types of cartilage dictate the capacity of the tissues to resist stress.

yaline cartilage also provides a very low frictional surface to accommodate joint movement. The ıysical properties of hyaline cartilage are a direct result of its extracellular matrix composition ıd organization.

Surgeons have developed alternative strategies to subchondral plate penetration in an effort deliver a more appropriate "hyaline" cell type to articular cartilage defects, and thus to achieve ore durable results. For example, autologous perichondral grafts from the rib have been used to eat full-thickness articular defects in the knee (Homminga *et al.*, 1990). Perichondrial grafts were it to fit the defects and were immobilized with fibrin glue in the defect site. By 1 year following ansplantation, 18 of 25 patients exhibited excellent functional recovery, and arthroscopic assess-ent indicated that the defects were completely filled with cartilage-like tissue in 90% of the de-cts. Unfortunately, long-term follow-up (up to 8 years) with these and other patients revealed at approximately 60% of the patients experienced treatment failure, which in many cases was a onsequence of endochondral ossification and graft delamination (Gillogly *et al.*, 1998).

O'Driscoll has utilized autologous periosteal tissue in full-thickness articular defects in an ef-ırt to achieve a more durable repair tissue (O'Driscoll *et al.*, 1986). This procedure involved de-ridement of the defect to a bleeding bed followed by suturing the periosteum to the base of the efect, with the cambium layer facing toward the joint space. Follow-up analysis of 15 patients re-ortedly treated with this procedure revealed that 9 patients experienced satisfactory results and 6 perienced graft failure within a few years. Other investigators that have utilized periosteal grafts ave reported similar results in that their clinical case studies (Angermann, 1994; Hoikka *et al.*, 990; Rubak *et al.*, 1982). These results have indicated lack of durability of the repair tissue in ıost patients and the available biopsy data have indicated that the repair tissue in the defect sites as not hyaline-like cartilage.

Another surgical approach to cartilage injury is osteochondral grafting. This approach in-olves the replacement of the entire bone and cartilage structure of the femoral condyle with size-natched human cadaver tissue (McDermott *et al.*, 1984). The reported clinical success observed sing this procedure for repairing osteochondral defects, coupled with the lack of adequate donor ssue, has led to the development of an alternative approach for repair of small cartilage defects nvolving the use of autologous cylindrical plugs of hyaline cartilage and underlying bone. These lugs are harvested from a minor weight-bearing area of the intercondylar notch or the anterior aperior lateral femoral condyle and are press-fitted into matching holes that have been introduced ı defects in the femoral condyle (Bobic, 1996; Matsusue *et al.*, 1993). Preliminary results from atients treated with this procedure suggest that it may be useful for the treatment of small artic-lar defects of less than 2 cm^2. However, no long term data are available from patients that have een treated using this procedure, and concern has been expressed regarding the durability of the epair tissue as well as the ultimate fate of the harvest sites.

AUTOLOGOUS CHONDROCYTE TRANSPLANTATION

Given the generally disappointing intermediate to long-term results obtained with the vari-ıus surgical procedures outlined above for the treatment of full-thickness articular defects, inves-igators explored alternative strategies to mediate a more durable repair. Because recruitment of ells from bone marrow was observed to induce formation of fibrocartilage, and transplantation of tissues such as perichondrium or periosteum to cartilaginous defects often led to ossification, it vas concluded that perhaps a more productive strategy was to isolate and utilize autologous artic-lar chondrocytes to mediate the repair process. This strategy was particularly appealing because rticular chondrocytes were the cell type normally involved in the production and maintenance of yaline cartilage on the articular surface.

Pioneering work by Benya and Shaffer (1982) had demonstrated that it was feasible to isolate abbit chondrocytes from articular cartilage and propagate them *in vitro*. The work of these in-restigators had demonstrated that once articular chondrocytes were enzymatically released from yaline cartilage and cultured on tissue culture plastic, they exhibited a profound change in their henotype, exhibited by development of a fibroblastic morphology and a switch in production rom type II collagen to type I collagen. Importantly, it was demonstrated that once the propa-ated chondrocytes were released from the tissue culture plastic surface and placed in suspension ulture, they went through a process of "redifferentiation" as judged by reexpression of type II col-agen and chondroitin sulfate proteoglycans.

Based on these observations it seemed feasible that isolation of chondrocytes from a small biopsy sample of articular cartilage, followed by enzymatic isolation, propagation of the cells, and ultimate reintroduction of the propagated cells into an articular defect, may provide a means to repair the defect with "hyaline-like" cartilage, rather than the fibrocartilage that was observed when cells recruited from bone marrow had been used to mediate the repair process. It seemed likely that if it were possible to produce hyaline-like cartilage in an articular defect, then the quality and durability of the repair tissue would be superior to that provided by fibrocartilage.

It is worth noting that the concept of autologous cell-based therapy for treatment of [illegible] tissue injury was initially developed and reduced to practice by Rheinwald and Green [illegible] Compton *et al.*, 1993; Green *et al.*, 1979). These investigators developed methods to isolate [illegible] atinocytes from small skin biopsies, propagate the cells to form epidermal grafts, and then use these grafts to provide permanent skin replacement for severe burn victims. This technology was commercialized in 1989 and has been successfully utilized by many surgeons for treatment of catastrophic burn victims.

Armed with this information investigators initiated experiments to test the possibility of using propagated articular chondrocytes for treatment of articular defects in animal models of articular cartilage injury. Grande *et al.* (1989) evaluated the utility of autologous chondrocyte implantation in a rabbit model of articular cartilage injury. A 3-mm-diameter chondral defect was introduced in the right and left patellas of the animals, chondrocytes were isolated from the cylindrical plugs of cartilage removed from the defect site, and cells were enzymatically released from the harvested tissue and propagated in tissue culture. Between 2 and 3 weeks following the initial surgery, a second surgery was performed to introduce the propagated chondrocytes into the defect site of the right knee. The patella of the left knee served as the control in these experiments. A periosteal patch (cambium layer side toward the defect) was sutured in place over the defects in both knees and approximately 1×10^6 cells were introduced underneath the patch of the right knee. Six weeks following implantation, the animals were sacrificed and their joints and defect sites were evaluated grossly and histologically. The results of this study revealed that grafted sites had significantly less synovitis and other degenerative changes as compared to control sites. Histologic evaluation of control sites indicated that there was an average of 18% fill of the control defects with repair tissue as compared to an average of 82% fill of the defects in cell-grafted sites. It was concluded based on histologic evaluation, that the repair tissue in the cell-grafted defects was very similar to the hyaline cartilage present in surrounding nonwounded articular cartilage. Results of other experiments using propagated chondrocytes that had been radiolabeled with tritiated thymidine provided evidence that the implanted cells were incorporated into the repair tissue and were contributing to the repair process.

During the same period of time that Grande *et al.* were studying autologous chondrocyte implantation in rabbits, Wakitani *et al.* (1989) evaluated the use of allogeneic articular chondrocyte transplantation for treatment of articular cartilage defects in the patellar groove of the right knee of rabbits. These studies were different from those of Grande *et al.* not only in the use of allogeneic versus autologous cells but also by the fact that the allogeneic cells were not propagated and were delivered in a collagen gel versus a cell suspension underneath a periosteal patch. Despite these differences, the results of these studies indicated that defect sites implanted with cells exhibited dramatically improved healing as compared to untreated defects or defects treated with the collagen gel alone. Hisrologic evaluation of cell implant sites indicated that the repair tissue was composed of hyaline cartilage.

Based on these promising results Brittberg *et al.* initiated clinical evaluation of autologous chondrocyte implantation in human patients using the basic procedure described by Grande (Fig. 49.2). The results of the first 16 patients treated with this therapy in femoral condyle defects and followed for 16–66 months are summarized in Table 49.2 (Brittberg *et al.*, 1994). The results indicated that two years after treatment, 14 of 16 patients of these patients experienced good to excellent clinical results. Clinical results for patients with patellar transplants were less impressive with only 2 of 7 patients experiencing excellent results. Biopsy specimens were obtained for 15 of the 16 patients with femoral condyle transplants. Histologic evaluation of these biopsy specimens indicated that the majority (11 of 15) contained hyaline-like cartilage (Fig. 49.3). Immunohistochemical analysis of 5 biopsy specimens with hyaline-like cartilage provided evidence of type II collagen staining.

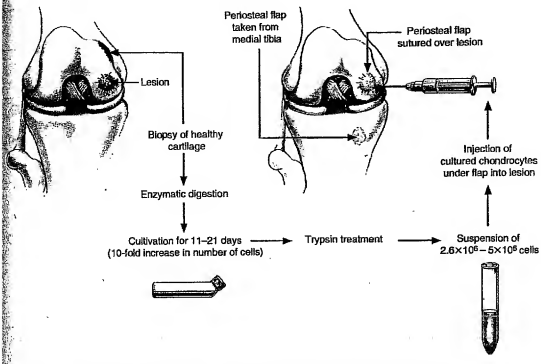

Fig. 49.2. Flow diagram for articular chondrocyte harvest and implantation, as performed in the treatment of deep cartilage defects in the knee with autologous chondrocyte implantation. From Brittberg et al. (1994). Copyright © 1994 Massachusetts Medical Society. All rights reserved.

COMMERCIALIZATION OF AUTOLOGOUS CHONDROCYTE IMPLANTATION

The preclinical and clinical results outlined above for autologous chondrocyte implantation, along with clear evidence of an unmet medical need in the area of clinical management of articular cartilage injury, stimulated interest in providing this treatment on a commercial basis, as had been done previously for autologous keratinocyte transplantation for severe burn victims. Conversion of the methods initially utilized by Brittberg *et al.* to a commercially feasible process presented many technical and quality control challenges. As noted above, autologous chondrocyte implantation relies on the use of cultured cells, initially isolated from a minor weight-bearing surface of the femoral condyle; the cells are expanded *in vitro*, and finally administered to the clinically relevant site for action. Because the biosynthetic profile of cultured animal chondrocytes was shown to be altered during proliferative expansion *in vitro* (Benya and Shaffer, 1982), an evaluation of the ability of propagated human chondrocytes subsequently to reexpress the appropriate cellular phenotype consistent with differentiated articular cartilage, as had been demonstrated for rabbit chondrocytes, was required to ensure the delivery of a functional and reproducible product.

Transcriptional analyses of human chondrocytes propagated on tissue culture plastic revealed that they underwent the reversible "dedifferentiation" process, as had been observed for other mammalian species, with reduction in expression of type II collagen and aggrecan and an up-regulation of type I collagen (Binette *et al.*, 1998). Release of the human chondrocytes from tissue culture plastic and subsequent culture in suspension culture resulted in a reexpression of type II collagen and aggrecan, but importantly showed no evidence of expression of type X collagen, a marker of chondrocyte hypertrophy and ossification (Fig. 49.4). The results of these experiments provided evidence that human chondrocytes, like the chondrocytes of other animal species, possessed the inherent capability to reexpress an articular cartilage phenotype following cellular propagation. This type of assay provided a useful means to assess the effects of various process changes on the capacity of cells to redifferentiate. For example, cultured autologous human articular chon-

Table 49.2. Femoral condylar defects in 16 patients treated with transplanted chondrocytes[a]

Patient No.	Age (years)/ sex	Duration of symptoms (years)	Size of defect (cm2)	Microscopic appearance	Histologic appearance	Biopsy (M.A.)[b]	Duration of follow-up (M.A.)[b]	Clinical grade[c]
1	27/M	3	1.6	Not BA	FH	16	16	Poor (2nd operation)
2	24/M	3	2.0	BA, CW	FH	14	48	Good
3	22/M	2	3.0	Not BA, CW	FH	12, 36	36	Poor (2nd operation)
4	48/M	3	2.0	BA, CW	FH	12, 24	48	Good
5	14/F	2	3.0	BA	HL	12, 46	46	Good
6	25/F	1	1.6	BA	HL	12	55	Excellent
7	40/M	3	2.2	BA	HL	22	59	Excellent
8	46/M	2	2.0	BA	HL	16	48	Good
9	22/F	3	4.0	BA	HL	12, 46	46	Excellent
10	26/M	3	2.4	BA	HL	12	36	Excellent
11	27/M	4	2.5	BA	HL	12	54	Good
12	27/F	2	2.0	BA	No biopsy	—	36	Good
13	23/M	6	5.0	BA	HL	17	24	Good
14	18/M	6	4.4	BA	HL	12, 32	32	Good
15	32/F	3	4.5	BA	HL	12	27	Excellent
16	19/M	2	4.0	BA	HL	12, 36	36	Excellent

[a]Patients 3 and 11 had injuries of the lateral femoral condyle, and all other patients had injuries of the medial femoral condyle. The follow-up od for patients 1 and 3 ended at the time of the second operation. Abbreviations: BA, biologically acceptable; FH, fibrous hyaline cartilage; CW, central wear; HL, hyaline-like cartilage.

[b]M.A., Number of months after transplantation.

[c]Note the correlation of clinical grade with the histologic appearance of repair tissue observed in patients treated with autologous chondrocyte plantation.

drocytes for clinical use were first propagated in culture medium composed of Ham's F-12 um supplemented with 15% (v/v) autologous serum (Brittberg *et al.*, 1994). Cells from each tient were cultured in medium supplemented with their own serum. The use of autologous man serum for propagation of chondrocytes was judged to be technically and economically infeasible. Therefore, the redifferentiation potential of adult articular chondrocytes cultured donor-matched autologous human serum (Brittberg *et al.*, 1994) or a single lot of commer

Fig. 49.3. Histologic section from a biopsy of cartilage repair tissue 36 months after treatment of an articular cartilage defect on the femoral condyle with autologous chondrocyte implantation. From Brittberg et al. (1994). *Copyright © 1994 Massachusetts Medical Society. All rights reserved.*

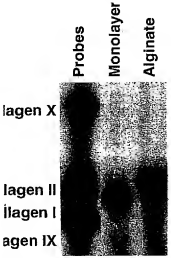

Fig. 49.4. Collagen types I, II, IX, and X gene expression during adult articular chondrocyte redifferentiation in long-term suspension culture. RNA isolated from human articular chondrocytes growing in a proliferative monolayer or in an alginate suspension was subjected to RNase protection assay using antisense RNA probes for types I, II, IX, and X collagen. From Binette et al. (1998).

ble bovine serum [Dulbecco's modified eagle's medium (DMEM) supplemented with 10% bovine serum] (Aulthouse *et al.*, 1989; Binette *et al.*, 1998; Bonaventure *et al.*, 1994) was ared. The study revealed that redifferentiation potential, or chondrocyte function, was more itent in medium supplemented with the single lot of fetal bovine serum (FBS) (Fig. 49.5). nconsistent maintenance of articular chondrocyte redifferentiation potential using matched · sera was the likely consequence of varying levels of growth and differentiation factors ob- l in different lots of serum (Freshney, 1994; Yaeger *et al.*, 1997).

iubsequent studies using numerous strains of human chondrocytes propagated with media emented with 10% bovine serum revealed that the age of the individual from which the chon- tes were isolated had a very modest effect on their potential to redifferentiate (Fig. 49.6). rise, it was observed that the number of population doublings expended prior to implanta- was unaffected by age (Fig. 49.7). Because chondrocytes for implantation are routinely pas- an average of 8 population doublings prior to clinical use, it is worth noting that chondro- retain their ability to redifferentiate until just before cell senescence, at about 35–50 lation doublings.

?rior to commercialization of autologous chondrocyte implantation, additional animal studere performed in an effort to better understand and document the process of articular carti- epair that was mediated by this cell-based therapy. Experiments in dogs revealed that redifftiation of autologous articular chondrocytes was observed within 6 weeks of implantation f-mm cartilage defects created in the femoral condyle or trochlear groove in the stifle joint of nimals (Shortkroff *et al.*, 1996). A chondrocyte nesting phenomenon was observed, where

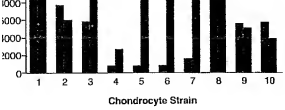

Fig. 49.5. Comparison of chondrocyte culture in autologous human serum vs. bovine serum. Effect of serum source on the ability of human articular chondrocytes to redifferentiate following proliferative expansion in monolayer. Human articular chondrocytes were cultured in culture medium supplemented with fetal bovine serum or donor-matched autologous human serum. Redifferentiation of chondrocytes was assessed by counting colonies in agarose suspension culture, as described by Benya and Shaffer (1982).

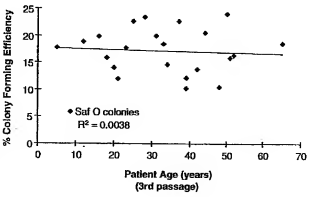

Fig. 49.6. Impact of patient age on the ability of human articular chondrocytes to redifferentiate in suspension culture following proliferative expansion in monolayer. The colony-forming ability of human articular chondrocytes derived from the articular cartilage of 22 donors ranging in age from 5 to 65 years was assessed following monolayer culture into the third passage (approximately 7–10 population doublings).

clusters of implanted chondrocytes in lacunae were located within type II collagen-positive matrix (Fig. 49.8). Chondrocytes having a fibroblastic morphology were observed in type I collagen-positive matrix. A transitional morphology was identified within the cartilage defect, whereby chondrocytic morphology was apparent in the depths of the defect and fibroblastic morphology was present toward the articular surface. Untreated defects without periosteum or cells, and defects treated with periosteum alone, exhibited cellular fill that was consistent with fibrocartilage.

At 6 months in the canine cartilage repair model, the tissue filling the cartilage defects was hyaline-like, with articular chondrocytes in lacunae (Shortkroff *et al.*, 1996). Columnar organization of chondrocytes was observed in some histologic sections (Fig. 49.9). Although a general trend toward more cartilage repair was observed at 6 months in the chondrocyte-implanted defects, the results were complicated by variable levels of spontaneous cartilage repair in the absence of chondrocyte implantation. The positive trend, in terms of improved cartilage repair in cell grafted sites as compared to controls, which was observed at 6 months, was no longer apparent at 12–18 months postimplantation (Breinan *et al.*, 1998). This was a consequence of both spontaneous healing in some of the control sites (no periosteum or cells) and development of degenerative joint disease in both control and treatment sites in many of the animals at these latter time points. Despite these limitations of the animal model, results of the study provided additional evidence for the safety of the procedure and provided insights into the mechanisms of chondrocyte-mediated repair in articular cartilage defects.

Autologous chondrocyte implantation was approved for treatment of articular cartilage defects by the Center for Biologics Evaluation and Research of the Food and Drug Administration in August, 1997, under provisions of accelerated approval guidelines. These regulations provide for approval of biological products for serious or life-threatening illnesses based on surrogate endpoints that are likely to predict clinical benefit. Approval of products under these regulations generally requires additional postapproval clinical studies to confirm that the surrogate end points utilized to provide the basis for approval of the product do indeed correlate with clinical outcomes. Such studies are currently in progress with autologous chondrocyte implantation.

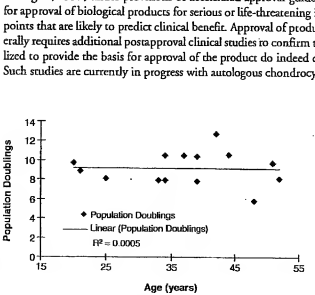

Fig. 49.7. The number of population doublings expended by human articular chondrocytes prior to clinical use. The number of population doublings was calculated as $\log_2$ (number of cells at subculture/number of cells seeded).

Fig. 49.8. Histologic section of an articular cartilage defect treated with cultured autologous chondrocytes at 6 weeks, stained for type II collagen. Autologous articular chondrocytes were implanted into 4-mm focal defects in the articular cartilage of adult dogs. Animals were sacrificed at 6 weeks and analyzed by histology. Note the type II collagen staining around the nests of chondroid cells (arrow pointing left) and in the calcified cartilage layer (arrow pointing down). Reprinted from Biomaterials, Vol. 17, No. 2. S. Shortkroff, L. Barone, H. P. Hsu, C. Wrenn, T. Gagne, T. Chi, H. Breinan, T. Minas, C. B. Sledge, R. Tubo, and M. Spector, pp. 147–154. Copyright 1996, with permission from Elsevier Science.

In addition to these studies, a patient registry program was voluntarily initiated by Genzyme Tissue Repair, the company responsible for commercializing autologous chondrocyte implantation (ACI). The registry consists of data collected at the cartilage harvest, cell implantation, and periodic follow-up visits to the physician at 6, 12, and 24 months. All patients treated with ACI have been included in the registry. When defects on the femoral condyles have been treated with ACI, significant improvements in joint function and patient symptoms compared to base line have been observed (>2 years of follow-up), with 86% of the patients recorded by themselves and their clinician as improved (Browne *et al.*, 1998; LaPrade and Swiontkowski, 1999).

In addition to these data, the initial positive results reported for femoral condyle defects by

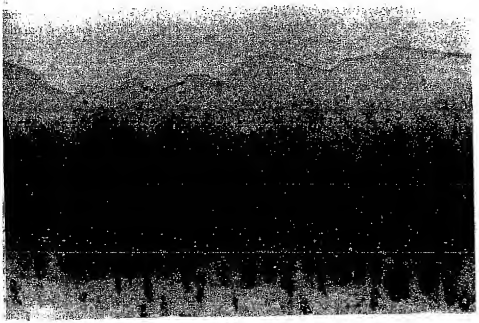

Fig. 49.9. Hyaline articular cartilage observed in a cartilage defect treated with cultured autologous chondrocyte implantation at 6 months. Animals were sacrificed at 6 months and histologic sections were stained with hematoxylin and eosin. Chondrocytes were observed in lacunae structures with columnar organization. This is characteristic of hyaline articular cartilage.

Brittberg *et al.* (1994) were further substantiated by a report on 219 patients at a 2- to 10-year follow-up visit (Peterson, 1998). Functional improvement was observed in 89% of cases with isolated femoral lesions. Moreover, histologic analysis of biopsies removed during second-look arthroscopy revealed that 74% of patients had hyaline-like articular cartilage repair. Good clinical outcome was correlated with hyaline-like repair tissue, supporting the hypothesis that biochemistry equals function in articular cartilage. The longevity of the ACI repair was also demonstrated, in that, 30 of the 31 patients (96%) who initially had good or excellent results at 2 years of follow-up maintained their good or excellent results at an average of 7.4 years postoperatively (Peterson, 1998). Additional reports from orthopedic surgeons have provided independent evidence that the procedure can be useful in the treatment of single large defects of up to 15 cm^2, or multiple defects of the femoral condyles (Gillogly *et al.*, 1998; Minas, 1998).

ALTERNATIVE STRATEGIES FOR THE DELIVERY OF CELL-BASED THERAPIES FOR CARTILAGE REPAIR

Despite the excellent clinical results with autologous chondrocyte implantation, opportunities for further improvement of this technology clearly exist. Patients who have been treated with autologous chondrocyte implantation do not typically return to full physical activity until at least 1 year postsurgery. The invasive nature of the surgical procedure, an open arthrotomy, and the time interval between chondrocyte implantation and production of a functional repair tissue have been hypothesized to be responsible for the relatively long period of patient rehabilitation. As noted above, the open arthrotomy, commonly known as an open-knee procedure, is required for the surgeon to suture the thin periosteal membrane directly to the margins of the cartilage defect. Therefore, strategies that facilitate the arthroscopic administration of a cartilage repair construct and at the same time accelerate the chondrocyte-mediated repair process are being investigated. The next-generation cartilage repair construct will likely be either a preformed cartilage tissue or will be composed of articular chondrocytes embedded within a biocompatible extracellular matrix supplemented with factors that positively impact chondrocyte growth and differentiation.

Articular chondrocyte viability and redifferentiation has been evaluated with several potential carrier matrices, including agarose (Rahfoth *et al.*, 1998), varying configurations of type I collagen gels and sponges (Ben-Yishay *et al.*, 1992; Frenkel *et al.*, 1997; Grande *et al.*, 1989, 1997; Qi and Scully, 1997; Wakitani *et al.*, 1989), type II collagen sponges (Nehrer *et al.*, 1998), hyaluronic acid derivatives (Solchaga *et al.*, 1999), poly(lactic acid), and poly(glycolic acid) and their derivatives (Freed *et al.*, 1994; Grande *et al.*, 1997; Vacanti *et al.*, 1991), and fibrin (Itay *et al.*, 1987; Sims *et al.*, 1998). The use of these materials to deliver articular chondrocytes to articular cartilage defects has resulted in varying degrees of cartilage repair in small animal models (Ben-Yishay *et al.*, 1992; Grande *et al.*, 1989; Itay *et al.*, 1987; Wakitani *et al.*, 1989). The results obtained in these models were complicated by varying levels of spontaneous repair, presumably due to penetration of the subchondral bone and the subsequent fibrocartilaginous response. However, the importance of cells in the repair of cartilage defects was illustrated by the fact that defects treated with extracellular matrix constructs containing cultured cells typically exhibited enhanced cartilage repair over control matrices without cells.

In terms of accelerating cell-mediated tissue repair, articular chondrocytes respond to a number of growth factors that stimulate proliferation and differentiation, including insulin, insulin-like growth factor I and II (IGF-I and IGF-II), and members of the TGF-β superfamily, including some of the bone morphogenetic proteins (BMP2 and BMP4), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (Martin and Buckwalter, 1996). Moreover, a positive impact on articular cartilage repair *in vivo* has been observed for several of these factors when supplemented into an extracellular matrix delivery vehicle including insulin-like growth factor-I into fibrin matrices (Fortier *et al.*, 1999), or members of the TGF-β superfamily into type I collagen constructs (Hunziker and Rosenberg, 1996).

The paradigm for preformed cartilage tissue was set by osteochondral allografts. Osteochondral allografts have been used successfully for the resurfacing or reconstruction of joints missing large surface areas (Bentley, 1992; Newo *et al.*, 1991). These bone and cartilage grafts have been shown to integrate very well with the existing bone and provide rapid clinical benefit. However, the supply of such tissue is extremely limited. Methods have been developed for the generation of a predifferentiated cartilage tissue *in vitro* (Kandel *et al.*, 1995; Peel *et al.*, 1998). The pre-differ-

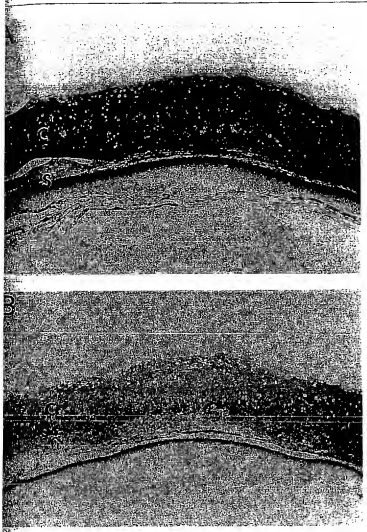

Fig. 49.10. Histologic appearance of a cartilaginous composite graft composed of articular chondrocytes and small intestine submucosa following 8 weeks in culture. (A) Cartilaginous tissue (C) on small intestine submucosa (S) stained with toluidine blue. (B) A zone of mineralization (arrows) is apparent in the lower area of the cartilaginous tissue (C) when cultured under conditions favoring bone formation. This may be helpful for integration with the subchondral bone in vivo. From Peel et al. (1998).

tiated cartilaginous implant can be produced *in vitro* by culturing articular chondrocytes on a pe II collagen-coated filter membrane, or on a thin membrane of small intestine submucosa kandel *et al.*, 1995, 1997; Peel *et al.*, 1998). After 4–6 weeks in culture a cartilage tissue con-ruct characterized by the histologic hallmarks of hyaline articular cartilage is observed (Fig. 9.10). This hyaline-like cartilage persists when implanted into cartilage defects *in vivo*. Integra-ion of the preformed cartilage implant with the cartilage surrounding of the defect is better than hat observed to subchondral bone.

CONCLUSIONS

Taken together, the preclinical and clinical data summarized above provide compelling evi-dence that chondrocyte implantation can provide superior clinical results in the treatment of ar-icular cartilage defects as compared to alternative cell-based strategies that either generate fibro-artilage or ultimately lead to ossification at the repair site. Scientifically, the use of articular hondrocytes to mediate repair of articular cartilage seems logical, just as it seems logical to use eratinocytes to replace epidermis following massive cutaneous injury. Despite the promising re-ults with autologous chondrocyte implantation, it is clear that significant opportunities for im-rovement of this procedure exist. As noted above, the current procedure is technically challeng-ng and results in a lengthy rehabilitation process. Thus, it is likely that the next-generation ell-based product to treat articular cartilage damage will be performed using minimally invasive

procedures and will be configured in a manner that will significantly reduce the time to gener functionally active tissue in the defect site. It is also clear that the ultimate product to treat art ular cartilage injury will be characterized by its ability to be used in large-scale joint resurfac and will have the added ability to mediate the wound repair process in a manner to reverse the d generative processes of osteoarthritis. Although such a product seems a distant fantasy, given t scientific progress that has been made in our understanding of cartilage maintenance and repa during the past 20 years, such a dream may be closer than we think.

References

Angermann, P. (1994). Osteochondritis dissecans of the femoral condyle treated with periosteal transplantation: A p liminary clinical study of 14 cases. *Orthop. Int.* 2, 425–428.

Aulthouse, A. L., Beck, M., Griffey, E., Sanford, J., Arden, K., Machado, M. A., and Horton, W. A. (1989). Expression o the human chondrocyte phenotype *in vitro*. *In Vitro Cell. Dev. Biol.* 25, 659–668.

Baumgaertner, M. R., Cannon, W. D., Jr., Vittori, J. M., Schmidt, E. S., and Maurer, R. C. (1990). Arthroscopic de bridement of the arthritic knee. *Clin. Orthop. Relat. Res.* 253, 197–202.

Bendey, G. (1992). Articular tissue grafts. *Ann. Rheum. Dis.* 51, 292–296.

Benya, P. D., and Shaffer, J. D. (1982). Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell (Cambridge, Mass.)* 30, 215–224.

Ben-Yishay, A., Grande, D. A., Menche, D., and Pitman, M. (1992). Repair of articular cartilage defects using collagen- chondrocyte allografts. *38th Annu. Meet. Orthop. Res. Soc.*, Washington, DC, p. 174.

Bert, J. M., and Maschka, K. (1989). The arthroscopic treatment of unicompartmental gonarthrosis: A five-year follow- up study of abrasion arthroplasty plus arthroscopic debridement and arthroscopic debridement alone. *Arthroscopy* 5, 25–32.

Binette, F., McQuaid, D. P., Haudenschild, D. R., Yaeger, P. C., McPherson, J. M., and Tubo, R. (1998). Expression of a stable articular cartilage phenotype without evidence of hypertrophy by adult human articular chondrocytes *in vitro*. *J. Orthop. Res.* 16, 207–216.

Bobic, V. (1996). Arthroscopic osteochondral autograft transplantation in anterior cruciate ligament reconstruction: A pre- liminary clinical study. *Knee Surg. Sports Traumatol. Arthroscopy* 3, 262–264.

Bonaventure, J., Kadhom, N., and Cohen-Solal, L. (1994). Reexpression of cartilage-specific genes by dedifferentiated hu- man articular chondrocytes cultured in alginate beads. *Exp. Cell Res.* 212, 97–104.

Breinan, H. A., Minas, T., Barone, L., Tubo, R., Hsu, H. P., Shortkroff, S., Nehrer, S., Sledge, C. B., and Spector, M. (1998). Histological evaluation of the course of healing of canine articular cartilage defects treated with cultured au- tologous chondrocytes. *Tissue Eng.* 4, 101–114.

Brittberg, M., Lindahl, A., Nilsson, A., Ohlsson, C., Isaksson, O., and Peterson, L. (1994). Treatment of deep cartilage de- fects in the knee with autologous chondrocyte transplantation. *N. Engl. J. Med.* 331, 889–895.

Browne, J. E., Fu, F. H., Mandelbaum, B. R., Micheli, L. J., and Moseley, B. (1998). Autogenous chondrocyte implanta- tion for treatment of articular cartilage knee defects 2–4 year multi-center experience. *Transa. European Society of Sports Traumatology, Knee Surgery and Arthroscopy, 8th Congr.*, Nice, France, p. 103.

Buckwalter, J. A., and Lohmander, S. (1994). Operative treatment of osteoarthrosis. Current practice and future develop- ment. *J. Bone Jt. Surg., Am. Vol.* 76-A, 1405–1418.

Compton, C. C., Hickerson, W., Nadire, K., and Press, W. (1993). Acceleration of skin regeneration from cultured ep- ithelial autografts by transplantation to homograft dermis. *J. Burn Care Rehabil.* 14, 653–662.

Dandy, D. J. (1991). Arthroscopic debridement of the knee for osteoarthritis. *J. Bone Jt. Surg., Br. Vol.* 73B, 877–888.

Fortier, L. A., Lust, G., and Nixon, A. J. (1999). Insulin-like growth factor-I enhances cell-based articular cartilage resur- facing. *Trans. 45th Annu. Meet. Orthop. Res. Soc.*, p. 58.

Freed, L. E., Grande, D. A., Lingbin, Z., Emmanual, J., Marquis, J. C., and Langer, R. (1994). Joint resurfacing using al- lograft chondrocytes and synthetic biodegradable polymer scaffolds. *J. Biomed. Mater. Res.* 28, 891–899.

Frenkel, S. R., Toolan, B., Menche, D., Pitman, M. I., and Pachence, J. M. (1997). Chondrocyte transplantation using a collagen bilayer matrix for cartilage repair. *J. Bone Jt. Surg., Br. Vol.* 79B, 831–836.

Freshney. (1994). "Serum-free Media. Culture of Animals Cells," pp. 91–99. Wiley, New York.

Friedman, M. J., Berasi, C. C., Fox, J. M., Del Pizzo, W., Snyder, S. J., and Ferkel, R. D. (1984). Preliminary results with abrasion arthroplasty in the osteoarthritic knee. *Clin. Orthop. Relat. Res.* 182, 200–205.

Gillogly, S. D., Voight, M., and Blackburn, T. (1998). Treatment of articular cartilage defects of the knee with autologous chondrocyte implantation. *J. Orthop. Sports Phys. Ther.* 28, 241–251.

Grande, D. A., Pitman, M. I., Peterson, L., Menche, D., and Klein, M. (1989). The repair of experimentally produced de- fects in rabbit articular cartilage by autologous chondrocyte transplantation. *J. Orthop. Res.* 7, 208–218.

Grande, D. A., Halberstadt, C., Naughton, G., Schwartz, R., and Manji, R. (1997). Evaluation of matrix scaffolds for tis- sue engineering of articular cartilage grafts. *J. Biomed. Mater. Res.* 34, 211–220.

Green, H., Kehinde, O., and Thomas, J. (1979). Growth of cultured human epidermal cells into multiple epithelia suit- able for grafting. *Proc. Nat. Acad. Sci. U. S. Am.* 76, 5665–5668.

Hoikka, V. E., Jaroma, H. J., and Ritsila, V. A. (1990). Reconstruction of the patellar articulation with periosteal grafts. 4-year follow-up of 13 cases. *Acta Orthop. Scand.* 61, 36–39.

Hommings, G. N., Bulstra, S. K., Bouwmeester, P. S., and van der Linden, A. J. (1990). Perichondral grafting for carti- lage lesions of the knee. *J. Bone Jt. Surg., Br. Vol.* 72B, 1003–1007.

Hunter, W. (1743). On structure and diseases of articulating cartilage. *Philos. Trans. R. Soc. London, Ser. B* 24B, 514–521.

Hunziker, e. B., and Rosenberg, L. C. (1996). Repair of partial-thickness defects in articular cartilage: Cell recruitment from the synovial membrane. *J. Bone Jt. Surg., Am. Vol.* **78-A**, 721–733.

Itay, S., Abramovici, A., and Nevo, Z. (1987). Use of cultured embryonal chick epiphyseal chondrocytes as grafts for defects in chick articular cartilage. *Clin. Orthop. Relat. Res.* **220**, 284–303.

Johnson, L. L. (1986a). Arthroscopic abrasion arthroplasty. *In* "Operative Arthroscopy" (J. B. McGinty, ed.), pp. 341–360. Raven Press, New York.

Johnson, L. L. (1986b). Arthroscopic abrasion arthroplasty historical and pathologic perspective: Present status. *Arthroscopy* **2**, 54–69.

Johnson-Nurse, C., and Dandy, D. J. (1985). Fracture-separation of articular cartilage in the adult knee. *J. Bone Jt. Surg., Br. Vol.* **67B**, 42–43.

Kandel, R. A., Chen, H., Clark, J., and Renlund, R. (1995). Transplantation of cartilagenous tissue generated *in vitro* into articular joint defects. *Artif. Cells Blood Substitutes Immob. Biotechnol.* **23**, 565.

Kandel, R. A., Boyle, J., Gibson, G., Cruz, T., and Speagle, M. (1997). *In vitro* formation of mineralized cartilagenous tissue by articular chondrocytes. *In Vitro Cell. Dev. Biol.* **33**, 174–181.

Kim, H. K., Moran, M. E., and Salter, R. B. (1991). The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. *J. Bone Jt. Surg., Am. Vol.* **73-A**, 1301–1315.

LaPrade, R. F., and Swiontkowski, M. F. (1999). Update on the clinical experience with autologous chondrocyte implantation. *JAMA, J. Am. Med. Assoc.* **281**, 876–878.

Martin, J. A., and Buckwalter, J. A. (1998). Articular cartilage aging and degeneration. *Sports Med. Arthroscopy Rev.* **4**, 263–275.

Matsusue, Y., Yamamuro, T., and Hama, H. (1993). Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. *Arthroscopy* **9**, 318–321.

McDermott, A. G., Langer, F., Pritzker, K. P., and Gross, A. E. (1984). Fresh small-fragment osteochondral allografts. *Clin. Orthop. Relat. Res.* **197**, 96–102.

Minas, T. (1998). Autogenous cultured chondrocyte implantation in the repair of focal chondral lesions of the knee: Clinical indications and operative techniques. *J. Sports Trauma* **20**, 1–13.

Nehrer, S., Breinan, H. H., Ashkar, S., Shortkroff, S., Minas, T., Sledge, C. B., Yannas, I. V., and Spector, M. (1998). Characteristics of articular chondrocytes seeded in collagen matrices *in vitro*. *Tissue Eng.* **4**, 175–183.

Nevo, Z., Robinson, D., and Halperin, N. (1991). The use of grafts composed of cultured cells for the repair and regeneration of cartilage and bone. *In* "Bone" (B. K. Hall, ed.), p. 123. CRC Press, Boca Raton, FL.

O'Driscoll, S. W., Keeley, F. W., and Salter, R. B. (1986). The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. *J. Bone Jt. Surg., Am. Vol.* **68-A**, 1131–1140.

Peel, S. A. F., Chen, H., Renlund, R., Badylak, S. F., and Kandel, R. A. (1998). Formation of a SIS-cartilage composite graft *in vitro* and its use in the repair of articular cartilage defects. *Tissue Eng.* **4**, 143–155.

Peterson, L. (1998). Autologous chondrocyte transplantation: 2–10 year follow-up in 219 patients. *6th Annu. Meet., Am. Acad. Orthop. Surg.*, New Orleans, LA.

Qi, W. N., and Scully, S. P. (1997). Extracellular collagen modulates the regulation of chondrocytes by transforming growth factor-beta 1. *J. Orthop. Res.* **15**, 483–490.

Rahfoth, B., Weisser, J., Sternkopf, F., Aigner, T., von der Mark, K., and Brauer, R. (1998). Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits. *Osteoarthritis Cartilage* **6**, 50–65.

Rand, J. A. (1991). Role of arthroscopy in osteoarthritis of the knee. *Arthroscopy* **7**, 358–363.

Rheinwald, J. G., and Green, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell (Cambridge, Mass.)* **6**, 331–343.

Rodrigo, J. J., Steadman, J. R., Silliman, J. F., and Fulstone, H. A. (1949). Improvement of full-thickness chondral defect healing in the human knee after debridement and microfracture using continuous passive motion. *Am. J. Knee Surg.* **7**, 109–116.

Rubak, J. M., Poussa, M., and Ristila, V. (1982). Effects of joint motion on the repair of articular cartilage with free periosteal grafts. *Acta Orthop. Scand.* **53**, 187–191.

Shortkroff, S., Barone, L., Hsu, H. P., Wrenn, C., Gagne, T., Chi, T., Breinan, H., Minas, T., Sledge, C. B., Tubo, R., and Spector, M. (1996). Healing of chondral and osteochondral defects in a canine model: The role of cultured chondrocytes in regeneration of articular cartilage. *Biomaterials* **17**, 147–154.

Sims, C. D., Butler, P. E., Cao, Y. L., Casanova, R., Randolph, M. A., Black, A., Vacanti, C. A., and Yaremchuk, M. J. (1998). Tissue engineered neocartilage using plasma derived polymer substrates and chondrocytes. *Plast. Reconstr. Surg.* **101**, 1580–1585.

Solchaga, L. A., Lundberg, M., Yoo, J. U., Huibregtse, B. A., Goldberg, V. M., and Caplan, A. I. (1999). Hyaluronic acid-based polymers in the treatment of osteochondral defects. *45th Annu. Meet., Orthop. Res. Soc.*, Anaheim, CA. 56.

Timoney, J. M., Kvitek, J. S., Bertuch, R. L., and Alexander, A. H. (1990). Arthroscopy update: 6. Arthroscopy in the osteoarthritic knee. Long-term follow-up. *Orthop. Rev.* **19**, 371–373.

Vacanti, C. A., Langer, R., Schloo, B., and Vacanti, J. P. (1991). Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. *Plast. Reconstr. Surg.* **88**, 753–759.

Wakitani, S., Kimura, T., Hirooka, A., Ochi, T., Yoneda, M., Owaki, H., Ono, K., and Yasui, N. (1989). Repair of rabbit articular surfaces with allografts of chondrocytes embedded in collagen gels (in Japanese). *Nippon Seikeigeka Gakkai Zasshi* **63**, 529–538.

Yaeger, P. C., Masi, T. L., de Ortiz, J. L., Binette, F., Tubo, R., and McPherson, J. M. (1997). Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. *Exp. Cell Res.* **237**, 318–325.